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**An investigation into the development of upland and lowland tadpoles of the  
Common frog (*Rana temporaria*) and Common toad (*Bufo bufo*) when cultured  
at different temperatures**

**by Giles Watson**

**A dissertation submitted in partial fulfilment of the requirements for the  
degree of Master of Science in Advanced Ecology**

**Department of Biological Sciences**

**University of Durham**

**1992**



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## Chapter 1.

### Introduction

The common frog, *Rana temporaria* has an extremely wide geographical distribution extending as far north as the Arctic circle through North and Central Europe to the Far East of Asia. It has been recorded at altitudes as high as 3000m above sea level in the Italian Alps although it does not live above the snow-line (Frazer, 1983). It is common throughout the British Isles with the exception of some outlying islands that remain uncolonised. The date of spawning varies markedly over this range from January in Brittany (Juszizyk and Zamachowski, 1965) to the end of May in Finland (Koskela, 1973) and as late as July in the Alps at an altitude of 2231m. Distinct differences in the date of spawning are also noticeable on a much more local scale ranging from January in the south-west to March in the north of England and the majority of Scotland (Cooke, 1977). The species is most active at dusk and the majority of its time is spent in damp herbage returning to the water to breed. During winter frogs hibernate in a variety of places including mud at the bottom of ponds, ditches or in compost heaps for example.

The geographical range of the common toad, *Bufo bufo*, is also large but not as extensive as that of the common frog. The limits of the distribution of *Bufo bufo* are 65° North to 34° North with a maximum elevation of 2000m. compared to 70° North and 43° North for *Rana temporaria* (Boulenger, 1898). The common toad is present throughout mainland England, Scotland and Wales though it is not present in Ireland.

The common frog terminates its period of hibernation when the external temperature reaches approximately 4°C and migrates to the spawning site, often passing through several ponds on its journey to a specific spawning pond. It is thought that the frogs are attracted to a particular pond by the odour of glycollic acid produced by the algae *Chlamydomonas pulsatilla* which forms the major food source for the future tadpoles (Savage, 1971).

Savage (1961) discovered a significant correlation between the date of spawning and the rainfall and temperature to which the frogs were subjected to several months before spawning and hypothesised that these two environmental variables have an indirect effect on spawning by stimulating algal growth and thus increasing glycollic acid production. The high levels of rainfall experienced during

the Spring wash plant nutrients out of the soil and thus increase algal production. However, this explanation cannot account for the relative synchronous emergence and spawning of common frog populations observed in Britain due to the unpredictability of the rainfall pattern. In addition there is no direct evidence available to suggest that there is a direct link between the proportion of algal metabolites and spawning in the common frog under natural conditions.

Beattie (1985) from investigations on the common frog in the Wear Valley has proposed that the adults become temperature sensitive in mid-February and emerge from hibernation 106 day °C after this point. Beattie measured the water temperature in a series of ponds over a four year period and found that the temperature decreased by 1°C for every 270m increase in altitude. This explanation accounts for the variation that Beattie observed in the date of spawning with altitude which was delayed by approximately 6 days per 100m increase in altitude as the temperature decreases as altitude increases and also explains annual differences in the spawning date. Frogs may therefore possess a circannual 'clock' which activates a cumulative temperature-sensing mechanism at a certain time of the year. The initiation of temperature sensitising may have evolved in response to local environmental conditions and therefore commence on different dates in different areas. An alternative possibility is that an endogenous rhythm controls emergence from hibernation and has become adapted to local environmental conditions. After reaching the breeding pond a mating pair will swim about in amplexus until spawning occurs, usually in approximately 10 to 20 cm of water. It is believed that when the sticky eggmass produced by the female touches the belly of the male this acts as a stimulus and the male proceeds to fertilise the eggs (Savage, 1934). Amplexus is then terminated and the male goes in search of another mate.

Migration in the common toad is under the control of an intrinsic temperature independent rhythm which acts in conjunction with temperature and rainfall (Heusser and Ott, 1968). Most toads become sexually mature after three years. Toads prefer to spawn in the deeper areas of ponds and lakes; in Windermere colonies of common toads have been reported from 5cm to 4-5m although the average depth is approximately 40cm (Frazer, 1983).

The frog eggmass is usually free floating and possesses open channels which allow water to circulate around the eggs for increased oxygenation. The jelly surrounding each egg acts as protection from predators and possesses insulating properties in addition to its role as a food source.

This ability to maintain the egg temperature slightly above that of the surrounding water may be an important adaptation enabling the eggs to survive low temperatures experienced during the night. Savage (1950) found that the eggmass could maintain a temperature that was, on average, 0.65°C above that of the surrounding water.

Beattie (1977) discovered that frog egg capsules in low conductivity ponds at altitudes above 200m were 241% larger than those in high conductivity ponds below 200m and therefore lost heat less quickly thus facilitating egg survival in highland ponds where lower temperatures are experienced.

The mechanism by which toads locate the breeding pond is unknown; it may be similar to that of the frog i.e. a chemical attractant, but other possibilities include attraction by the calling of the first arrivals who have hibernated nearby or perhaps simply the fact that the toads know and recognise their home territory.

If the temperature suddenly falls when the toads are *en route* to the breeding site they will stop and dig in while any already present at the pond will seek shelter amongst vegetation or burrow into the mud at the bottom of the pond.

After a male and female have paired they swim around in amplexus the female releasing eggs every few minutes which are immediately fertilised by the male. The pair swim in and out of vegetation as the eggs are laid so that they become entwined, the whole process taking several hours.

The breeding date can be correlated with that of the common frog; the interval between the two varying with the time of first frog spawning. The mean date for toad spawning in Britain is 22 March.

In both frogs and toads the heart, blood vessels and gills develop as the embryo grows within the egg and possess a fine network of capillaries to enable gaseous exchange between blood and water. The mouth and gut also develop in association with the gills.

At the time of hatching the tadpole possesses a glandular area on the head which secretes a sticky material facilitating attachment to aquatic vegetation during the first few days of life. Toad eggs hatch after about 10 days under normal conditions and the tadpole is still embryonic; external gills are present but these degenerate within a few days (Frazer, 1983). The tadpoles feed on algae and its associated invertebrate populations until metamorphosis occurs. The eggs of the common toad

are smaller than those of the frog and they leave the mucilage at stage 15 or 16 compared to stage 20 for the frog.

Initially, during the herbivorous phase of their life cycle, the tadpole has beak-like mandibles surrounded by horny teeth to rasp at algae although it has been hypothesised that the actual nutrients acquired come from diatoms that feed on the algae (Frazer, 1983).

As the tadpole develops surface tissue grows over the external gills whose function is then replaced by internal gills which develop inside the branchial arches. As this process continues the branchial clefts which previously allowed water to be expelled are covered by tissue and now the tadpole must gargle each mouthful of water around the gill chamber to allow oxygen diffusion. A spiracle develops on the left hand side of the head to enable this water to be expelled.

The hind legs are the first to become visible as the front pair develop underneath the tissue covering the gill arches. As the gills degenerate they produce a secretion that digests some of the overlying tissue on the right hand side of the head thus allowing the front limb to protrude whilst on the left hand side of the body the front limb pushes through the spiracle. Lungs have now taken over the respiratory function from the gills.

The final external change before metamorphosis is complete is the reabsorption of the tail which provides nutrients for limb development and internal changes to the circulatory system to supply the new respiratory organs; the skin and lungs as the tadpole has stopped feeding by this stage. Although metamorphosis is associated with resorption of the tail to provide the required protein it will only take place if adequate animal protein is present in the diet. At the time of metamorphosis only approximately 1% of the tadpoles that hatched are still alive.

Frog skin is permeable to water and the passage of water across the skin is governed to some extent by the humidity of the surrounding air and thus frogs are confined to moist habitats. Toad skin, by contrast, is dry and thick and the passage of water is regulated by hormones produced in the pituitary gland and thus the toad is not restricted to damp environments to the same extent as the frog.

Toad tadpoles are extremely distasteful to many of their predators and therefore their survival rate in the wild is probably much better than that of the frog.



Female frogs leave the spawning site soon after breeding and travel further than the males who leave a couple of weeks later (Hazelwood, 1969). In Northern England the frogs are present in the herbage surrounding the pond by July but have dispersed further by August. By the end of September and beginning of October they return to their hibernation site which may be the pond from which they first departed. In Poland this migration is associated with a drop of approximately 10°C in air and water temperature (Kowalowski 1974). The immatures hibernate approximately two weeks after the adults. The adults feed on land taking any invertebrate of the appropriate size and therefore their diet changes with the seasonal abundance of different insects such as flies, slugs and snails. They do not, however, feed during the breeding season.

Toads spend the summer daytime in holes, under stones or buried in earth emerging to actively feed on rainy nights when the temperature exceeds 11°C. The toad diet is similarly broad to that of the frog with ants being a particular favourite.

In Poland, when the temperature is 7-15°C, toad spawn hatches in 7 to 10 days and metamorphosis occurs after 56 to 74 days and therefore the toadlets leave the spawning pond by mid June (Kowalowski 1974).

Temperature has an important effect on the distribution of frogs and toads. The geographical distribution of the genus *Rana* is closely related to its temperature tolerance. For example, if we look at four species of North American frogs; *Rana clamitans*, *R. palustris*, *R. pipiens* and *R. sylvatica* we find that *R. sylvatica* which has the northernmost geographical limit is also the most cold-tolerant. An additional correlation with distribution is that frogs that breed at low environmental temperatures have low minimum and maximum temperatures for breeding and for embryonic development too (Moore, 1939). Physiological differences in response to temperature also occur within species such as *R. pipiens* which cover an unusually wide geographic range; embryos of northern species develop faster at lower temperatures than southern species (Moore, 1949a).

Ruibal (1955) discovered variation in the rate of embryonic development of embryos of *R. pipiens* from different altitudes in Mexico; those from high altitude sites (3000m) developed slower at all temperatures than those from lowland sites (350m) even though the temperature at the high altitude sites was approximately 13°C lower throughout the year; the explanation was that the frogs

in lowland ponds were subject to more intensive interspecific competition and therefore the selection pressure for faster development was greater on this population.

An inability to adapt the embryonic development rate may therefore limit the distribution of an amphibian and this is thought to explain why geographically separated populations of *R. catesbeiana* and *B. valliceps* display no significant difference in the rate of their embryonic development at different temperatures (Moore, 1942, Volpe, 1957).

*Rana temporaria* has a wide distribution and therefore one would expect to observe variation in its embryonic development rate at different latitudes and indeed this is found, particularly at lower temperatures. At 8°C embryos from Northern England (latitude 55°) develop 21% faster than those from Belgium and France (latitude 52° to 45°) and 29% faster than those from Japan (latitude 41°) and thus embryos from northern latitudes develop faster at lower temperatures than those from southern latitudes and this is presumably an adaptation to the lower environmental temperature and shorter growing season experienced at higher latitudes (Beattie, 1977).

Douglas (1948) studied common frogs and common toads breeding near London. He found that as the temperature increased from 13.9 °C to 24.0 °C the time taken from first cleavage to stage 20 decreased. He also noted that the time taken between stages 3 and 20 at 17.6 °C was shorter with *R. temporaria* (97 hours) than with *B. bufo* (139 hours).

Beattie (1987) measured the development rates of lowland (mean altitude 187.0m) and upland (mean altitude 621.2m) embryos and found that those from lowland ponds developed approximately 1% faster at 16°C but 4% slower at 6°C; this may be an adaptation for the later spawning that occurs at higher altitudes (to avoid frost) and the lower pond water temperatures experienced during the embryonic development period. *R. temporaria* froglets leave lowland ponds in June/July in Northern England and upland ponds in August/September compared to late May or early June in most other parts of England (Smith, 1969). A faster rate of embryonic development may therefore compensate for the shorter growing season enabling the frog to acquire the energy reserves necessary to survive during winter.

Previous studies of frog and toad development rates with temperature have been terminated at stage 20 of the development cycle because following hatching, which occurs at this stage in the common frog and approximately four stages earlier in the Common toad, numerous additional

factors such as food (D'Angelo, Gordon and Charipser, 1941), crowding (Adolf, 1931; Richards, 1958, 1962; Rose, 1960; Akin, 1966) and iodine concentration (Lynn and Brambel, 1935) can affect the rate of development and time of metamorphosis.

The relationship between temperature and development is simple, 'within the permissible temperature range an increase in temperature speeds development' (Cossins and Bowler, 1987). Within the temperature range an animal can tolerate the rate of oxygen consumption usually increases in a fairly regular manner with increasing temperature. Generally a 10°C rise in temperature results in a doubling of oxygen consumption. The increase in rate caused by a 10°C rise in temperature is called the  $Q_{10}$  and if it is equal to 2 then oxygen consumption doubles over this 10°C increment.

*relationship between O<sub>2</sub> consumption and temperature*

The aim of the present study was to record the development rate of tadpoles cultured at different temperatures after hatching and through to metamorphosis. The potential problems previously described when culturing tadpoles beyond stage 20 were minimised as much as possible through careful experimental design and husbandry techniques.

Thus tadpoles of upland frogs, upland toads, lowland frogs and lowland toads were cultured at different temperatures and their development rates recorded to see if any significant differences were apparent and, if so, hypothesise on the possible adaptive significance of such differences and thus their potential evolutionary value.

The study was primarily concerned with comparing upland and lowland toads in order to hypothesise on possible factors that influence their development and thus may limit their altitudinal distribution. Upland and lowland frogs were also studied as these are known to have a much more extensive altitudinal distribution and thus direct comparisons between the two populations could be made.

## Chapter 2

### Materials and Methods

#### 2.1. Collection Sites

Tadpoles and spawn were collected from two ponds on 1 May and a third on 13 May. Table 1 shows the type of tadpoles taken from each site together with the altitude and national grid reference of the ponds and the date on which the spawn/tadpoles were collected.

Table 1. The type of tadpoles collected at particular sites.

Pond	Type of tadpoles collected	Date of collection	Development stage of tadpoles	National grid reference of pond	Altitude of pond (metres)
A	Toads	1 May	24	NZ 224421	60
B	Frogs	13 May	31	NZ 224421	90
C	Frogs and toads	1 May	18 and 14	NZ 064414	225

Frogs and toads spawn later at higher altitudes and the samples taken from the high altitude pond were still in the embryonic state when collected. Those from the two lowland ponds, however, had already hatched.

#### 2.2. Rearing Regime

Approximately 500 individual frog or toad embryos/tadpoles were collected from each site and returned to the laboratory. Pond C was the only one to contain both frogs and toads and therefore samples of each were taken.

For the purposes of this experiment upland is defined as an altitude above 200m and lowland below 100m.

Four different populations were therefore available for analysis; upland frogs, upland toads, lowland frogs and lowland toads.

The development stage of the embryos/tadpoles in each of the four populations was then identified and fifty individuals that were all at the same stage were taken and placed in plastic

transparent aquariums 40cm wide, 30cm deep and 30cm high. These were filled with 1500 cm<sup>3</sup> of tap water which had been left standing for approximately 1 week to allow any chlorine present to diffuse out. Five such aquariums were prepared for each of the four populations one of which was placed in a constant temperature room at 5°C, 8°C 10°C, and 15°C and a constant temperature oven at 25°C. These five different temperature environments all experienced the same 18 hour photoperiod which approximates to mid-summer daylength.

The water was changed every week and approximately every two days the tadpoles were fed. Initially 0.5g of nettle extract per tadpole was added to the aquariums but when they reached stage 40 this was changed to 0.5g catfood per tadpole as by this time the tadpoles are carnivorous. The water in the aquariums was changed and the feeding interval increased if the water became discoloured due to excess food causing deoxygenation and thus fouling of the water.

The aquariums were then observed every other day and a record taken of the number of individuals still alive and the development stage that each of these had attained. The development was classified into stages using those identified for the Gulf Coast Toad, *Bufo valliceps*, by Limbaugh and Volpe (1957). Those used are summarised in Table 2. which describes the characteristic features of each stage.

Table 2. The characteristics of the development stages of the Gulf Coast Toad, *Bufo valliceps*. (from Limbaugh and Volpe 1957).

Stage number	Stage description
14	Neural fold forms
15	The neural folds grow together
16	The neural fold closes to form the neural tube
17	The tail bud develops
18	Muscular movement can be observed
19	The heart starts beating
20	Gill circulation commences
21	The cornea is transparent allowing the underlying lens to be observed
22	Blood corpuscles circulate in the tail fin
23	The opercular fold covering the gills forms on each side
24	The operculum closes on the right-hand side
25	The operculum closes on the left-hand side
26	The limb buds appear; their length is less than one half of their diameter
27	The limb bud is equal to or greater than one half of its diameter

Table 2. cont.

28	The limb bud is equal to or greater than its diameter
29	The limb bud is equal to or greater than one and a half times its diameter
30	The limb bud is bullet shaped and its length is twice its diameter
31	The distal end of the limb bud is paddle-shaped
32	Indentation between toes 5-4
33	Indentation between toes 5-4; 4-3
34	Indentation between toes 5-4; 4-3; 3-2
35	Slight indentation between toes 2-1
36	Toes 1 and 2 joined; other separated
37	Five toes separated; no metatarsal tubercle
38	Metatarsal tubercle apparent
39	Pigment-free patches where subarticular tubercles will form
40	The subarticular tubercles appear
41	Transparent cover (skin window) over forelimb
42	One or both forelimbs protrude. Angle of mouth anterior to nostril
43	Angle of mouth between nostril and midpoint of eye
44	Angle of mouth between midpoint and posterior margin of eye
45	Angle of mouth at posterior margin of eye. Tail is a stub
46	Metamorphosis complete. Tail completely reabsorbed

The mean stage reached by each population at these time intervals was then calculated and these results are shown in Appendix A with the associated standard errors in Appendix B. The mean values could then be compared using a t-test.

The data for the survival rates of the four populations can be seen in Appendix C. Survivorship curves have been constructed from this data and compared using Peto and Peto's Logrank Test (Pyke and Thompson, 1986). The formula for this is as follows:

$$\text{Logrank} = ((d_1 - E_1)^2 / E_1) + ((d_2 - E_2)^2 / E_2)$$

d1= total number dead in population 1

d2= total number dead in population 2

E1= expected number of deaths in population 1 based on the total mortality in each time interval and the proportion surviving.

E2= expected number of deaths in population 2 based on the total mortality in each time interval and the proportion surviving.

The logrank value obtained is tested for significance using Chi-squared tables. The critical value for Chi-squared with 1° of freedom is 3.84 and therefore logrank values that equalled or exceeded this figure indicated a significant difference between the survival curves of the two populations under comparison.

### 2.3. Pond Temperature Records

A Cambridge temperature recorder was used to record the daily temperature fluctuations in water temperature of a pond in Durham City over a two week period. and from <sup>these</sup> ~~this~~ data the time at which the water reached it's mean daily temperature was estimated and at this time the water temperatures of ponds A, B and C were recorded. Although there is a large difference in the size of the ponds under investigation and that at which the Cambridge recording was made, the probe was only 10cm below the water surface at which depth the possible insulating properties of a large body of water that would reduce temperature fluctuations are likely to be negligible.

## Chapter 3

### Results

#### 3.1. Development Rates

The mean stages reached by frog and toad tadpoles at given time intervals are shown in Appendix A from which it can be seen that not all of the tadpoles have completed the same developmental stages. Obviously comparisons can only be made between populations that have completed equivalent stages and therefore it is these data that have been used to construct the summary tables in the following results section. These show the mean stages achieved by tadpole populations from the same initial stage after a given number of days.

Development rates were compared between upland frogs and toads, lowland frogs and toads, upland and lowland frogs and upland and lowland toads. The primary aim of such comparisons was to observe if the development rate of upland and lowland toads displayed any significant differences and, if so, how this may be related to their altitudinal distribution. Upland and lowland frogs were also used to provide a direct comparison with the upland and lowland toads.

The length of time over which the comparisons of mean development stage are made in Tables 3 to 8 are different in each table. This is because the length of time over which the two populations overlapped in the development stages completed varied depending upon the particular population under investigation and the incubation temperature of the culture.

##### 3.1.1. A Comparison Between the Development Rates of Upland Frogs and Toads at Different Temperatures

Table 3. shows the mean stages reached by upland frogs and toads developing at a series of different constant temperatures after 27 days. The mean starting stage was 18 for all the cultures and it can be seen that the mean development rates increased progressively from 5°C to 25°C for both frogs and toads.



Table 3. The mean stage  $\pm$  standard error reached by upland frogs and toads at different temperatures after 27 days. The starting stage was 18 in all cases. n is the number of tadpoles surviving to 27 days.

	Temperature ( $^{\circ}\text{C}$ )									
	5 $^{\circ}\text{C}$		8 $^{\circ}\text{C}$		10 $^{\circ}\text{C}$		15 $^{\circ}\text{C}$		25 $^{\circ}\text{C}$	
	n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE
Upland frogs	24	27.0 $\pm$ 0	19	27.0 $\pm$ 0	36	28.0 $\pm$ 0	12	29.6 $\pm$ .16	19	32.4 $\pm$ .20
Upland toads	5	19.0 $\pm$ 0	22	26.7 $\pm$ .10	16	27.5 $\pm$ .13	43	31.0 $\pm$ 0	16	40.8 $\pm$ .79

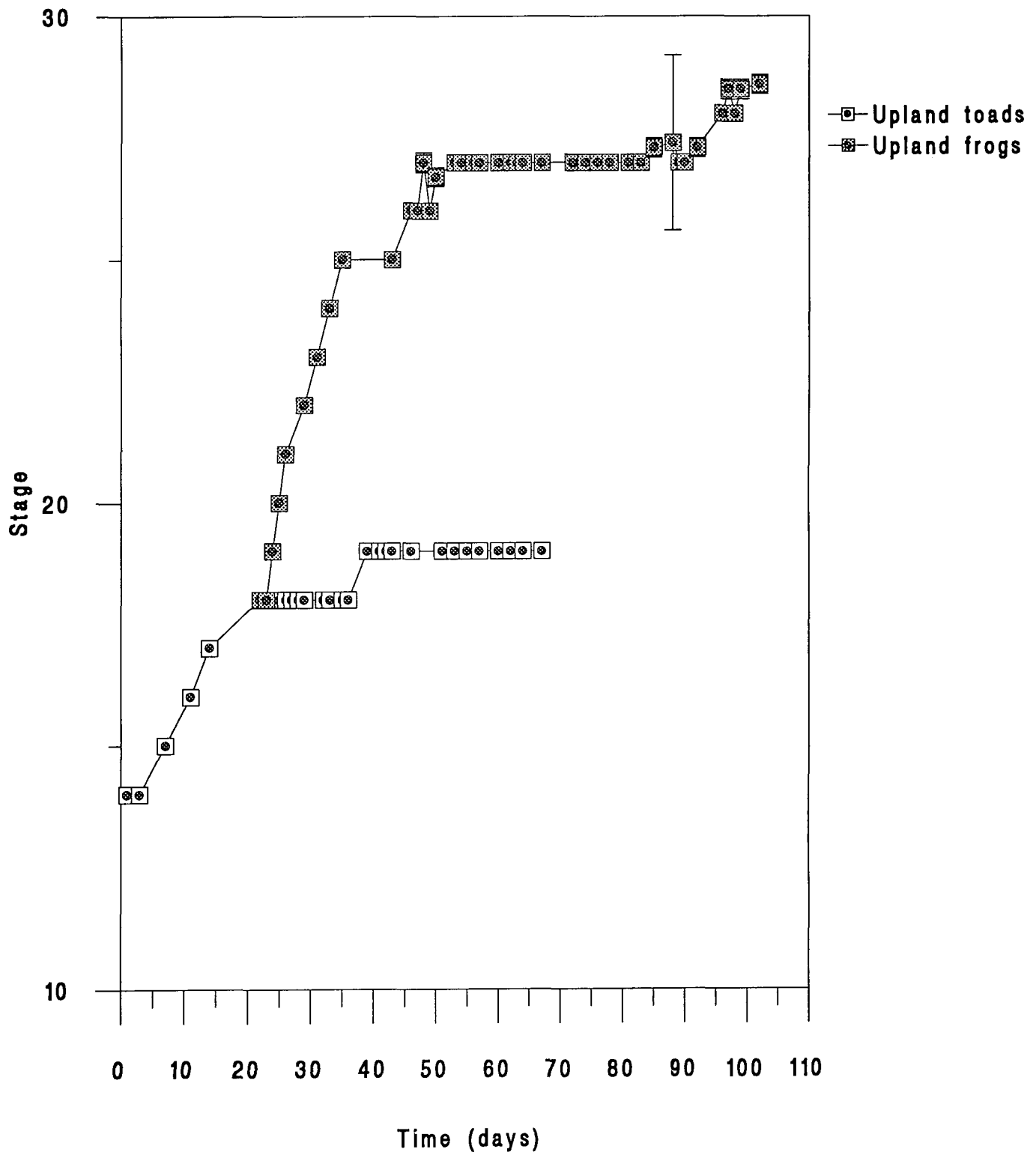
Table 4. compares the mean stage reached by upland frogs and toads after 27 days using a t-test and gives the p-value that this calculation represents. It also shows which animal was more advanced at the time when the recordings were taken and by how many stages.

Table 4. A comparison between the stages reached by upland frogs and toads at each temperature

Temperature ( $^{\circ}\text{C}$ )	Difference in mean stage	t-value	Degrees of freedom	p value
5 $^{\circ}\text{C}$	Frog +8.0	Infinite	27	<0.05
8 $^{\circ}\text{C}$	Frog +0.3	3.00	39	<0.05
10 $^{\circ}\text{C}$	Frog +0.5	3.85	50	<0.05
15 $^{\circ}\text{C}$	Toad +1.4	8.75	53	<0.05
25 $^{\circ}\text{C}$	Toad +8.4	10.31	33	<0.05

Figure 1. shows the development curves of upland frogs and toads at 5 $^{\circ}\text{C}$  at which temperature the upland frogs develop faster than upland toads. The curve shows that both upland frogs and toads initially develop rapidly but this development then slows down. This phenomenon of rapid initial development is displayed by many of the cultures and may indicate that the culture

Figure 1. The Mean Development Stage of Upland Frogs and Toads at 5 °C With Standard Error Bars Giving 95% Confidence Limits



regime is not as favourable to their development as the conditions that they were experiencing in the wild. There are several factors which could have a detrimental effect on the growth observed in the cultures such as overcrowding, competition or light regime and these possibilities are discussed later.

Figure 2. shows the development curves of upland frogs and toads at 8°C where the two populations display very similar development curves. However, Table 4. shows that after 27 days the upland frogs are 0.3 of a stage significantly more advanced than the upland toads.

Figure 3. shows the development curves of upland frogs and toads at 10°C. The two populations initially follow very similar development patterns. However, at approximately day 50 the frogs begin to develop more rapidly. Table 2. confirms that after 27 days the frogs are significantly more advanced than the toads and this gap is approximately half a stage.

Figure 4. shows the development curves of upland frogs and toads at 15°C. The upland toad population displays the most rapid development and are 1.4 stages significantly more advanced than the frogs after 27 days as can be seen from Table 2.

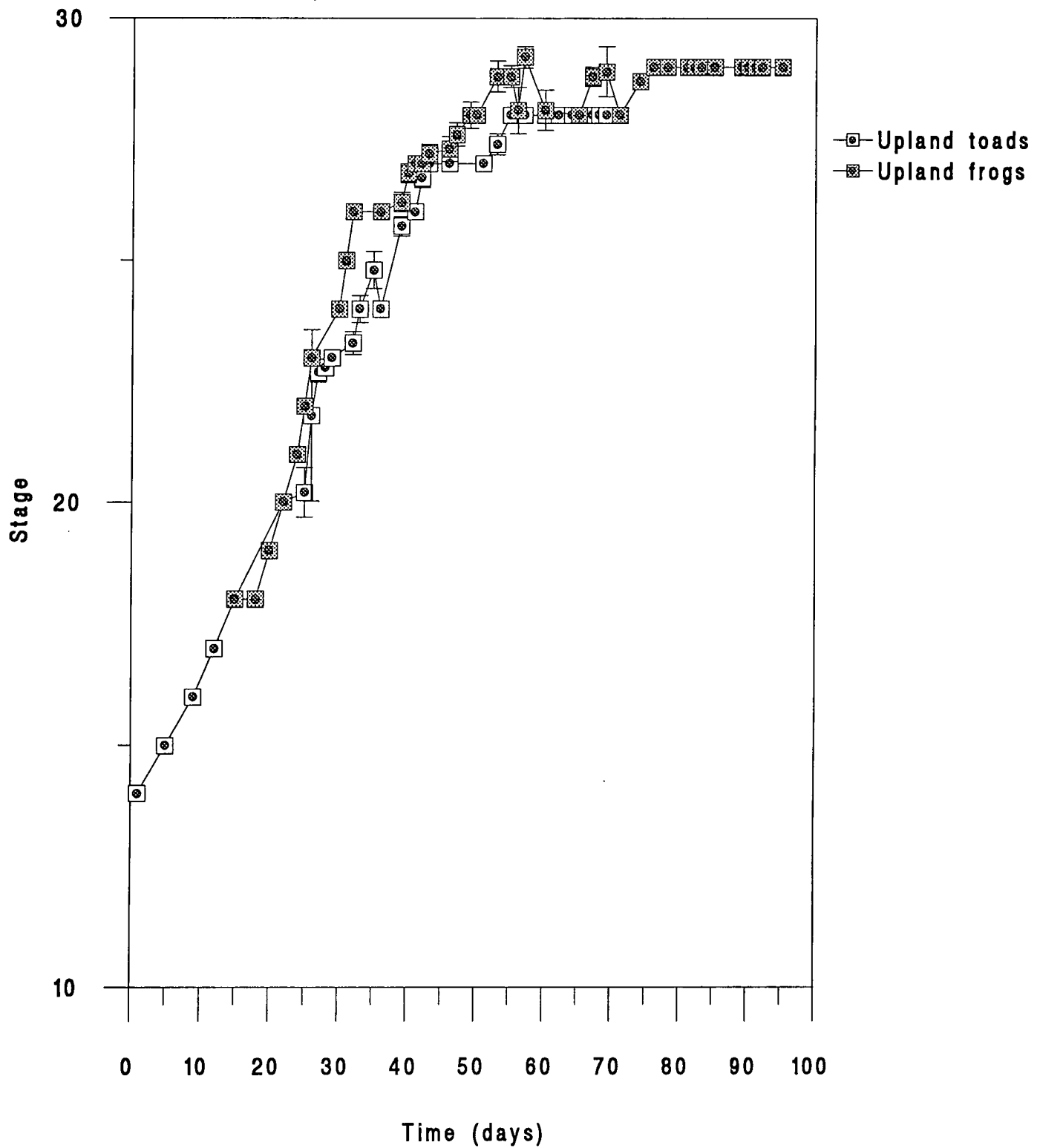
Figure 5. shows the development curves of upland frogs and toads at 25°C. The pattern observed at 15°C is continued and the upland toad population are clearly developing much faster and have completed metamorphosis when the upland frog population are only at approximately stage 33. Table 2 shows that this difference is significant after 27 days.

In conclusion, upland frogs developed faster than upland toads between 5°C and 10°C whereas upland toads grew more rapidly from 15°C to 25°C.

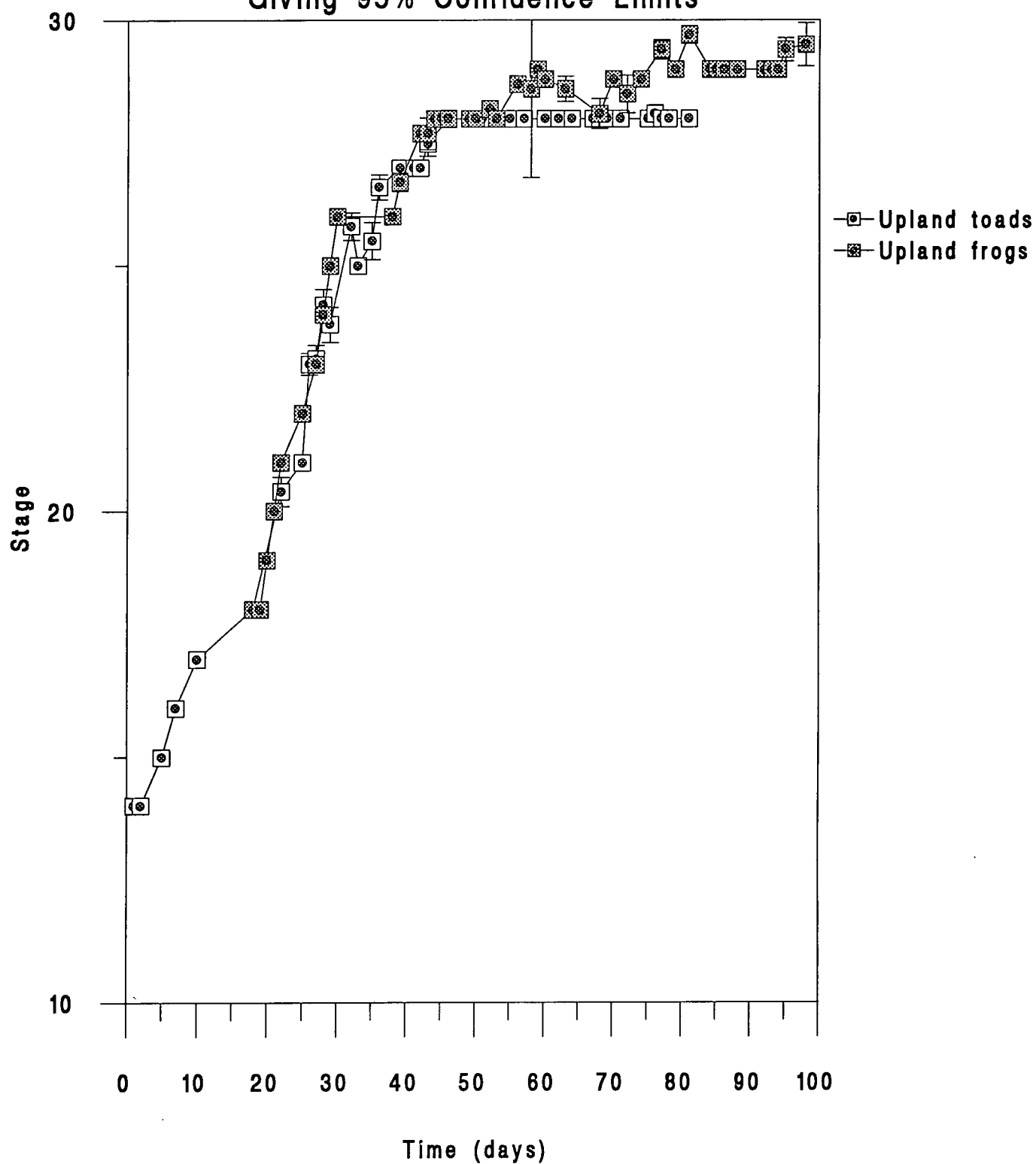
### 3.1.2. A Comparison Between the Development of Lowland Frogs and Toads at Different Temperatures

Table 5. shows the mean stages reached by lowland frogs and toads developing at a series of different constant temperatures after 42 days. The mean starting stage was 31 for all the cultures and it can be seen that the development rate of lowland frogs decreased from 15°C to 25°C but increased over this temperature range for lowland toads. Data is not included for development between 5°C and 10°C because there was no overlap in development stages completed over this temperature range.

Figure 2. The Mean Development Stage of Upland Frogs and Toads at 8 °C With Standard Error Bars Giving 95% Confidence Limits



**Figure 3. The Mean Development Stage of Upland Frogs and Toads at 10 °C With Standard Error Bars Giving 95% Confidence Limits**



**Figure 4. The Mean Development Stage of Upland Frogs and Toads at 15°C With Standard Error Bars Giving 95% Confidence Limits**

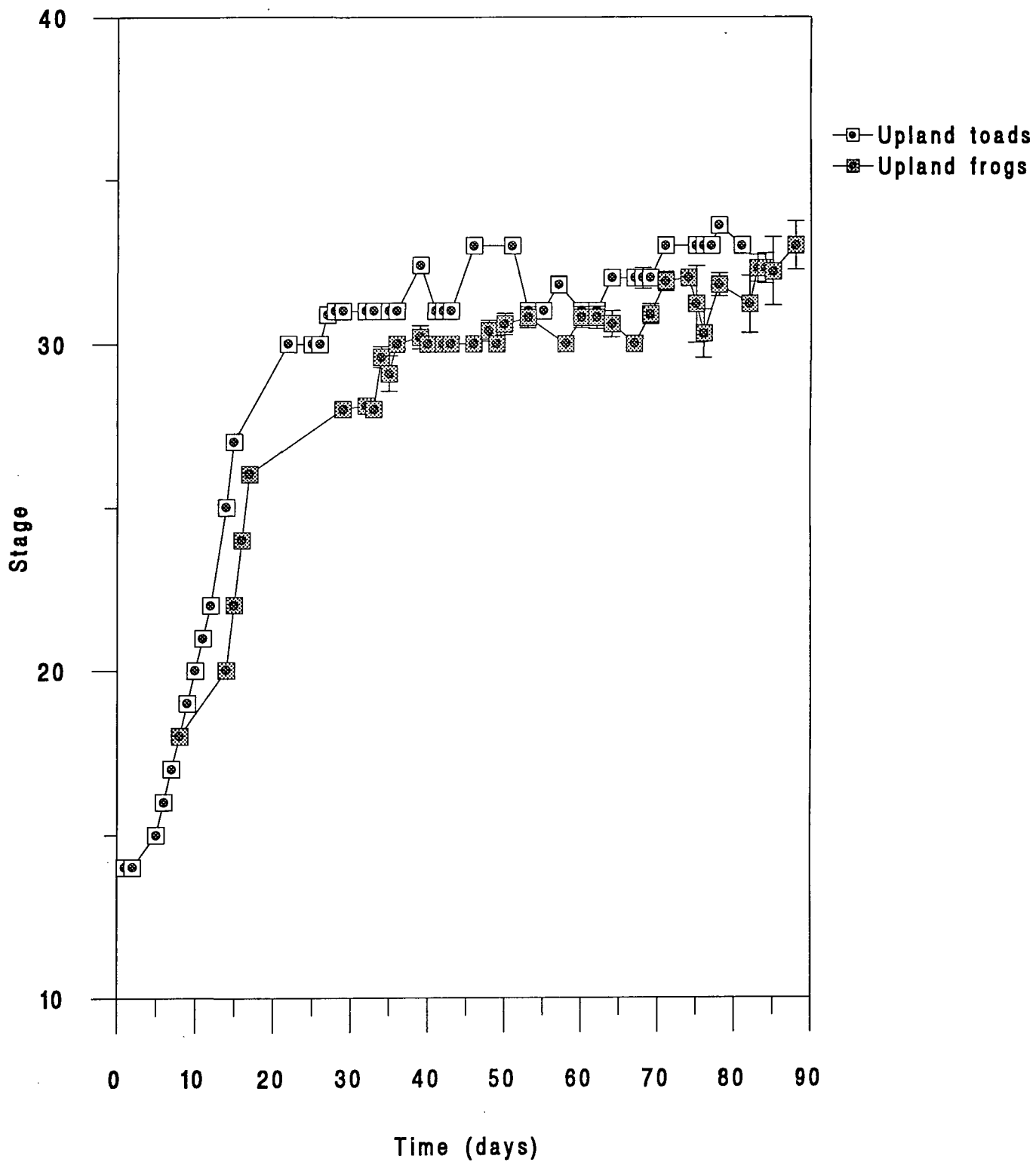


Figure 5. The Mean Development Stage of Upland Frogs and Toads at 25 °c With Standard Error Bars Giving 95% Confidence Limits

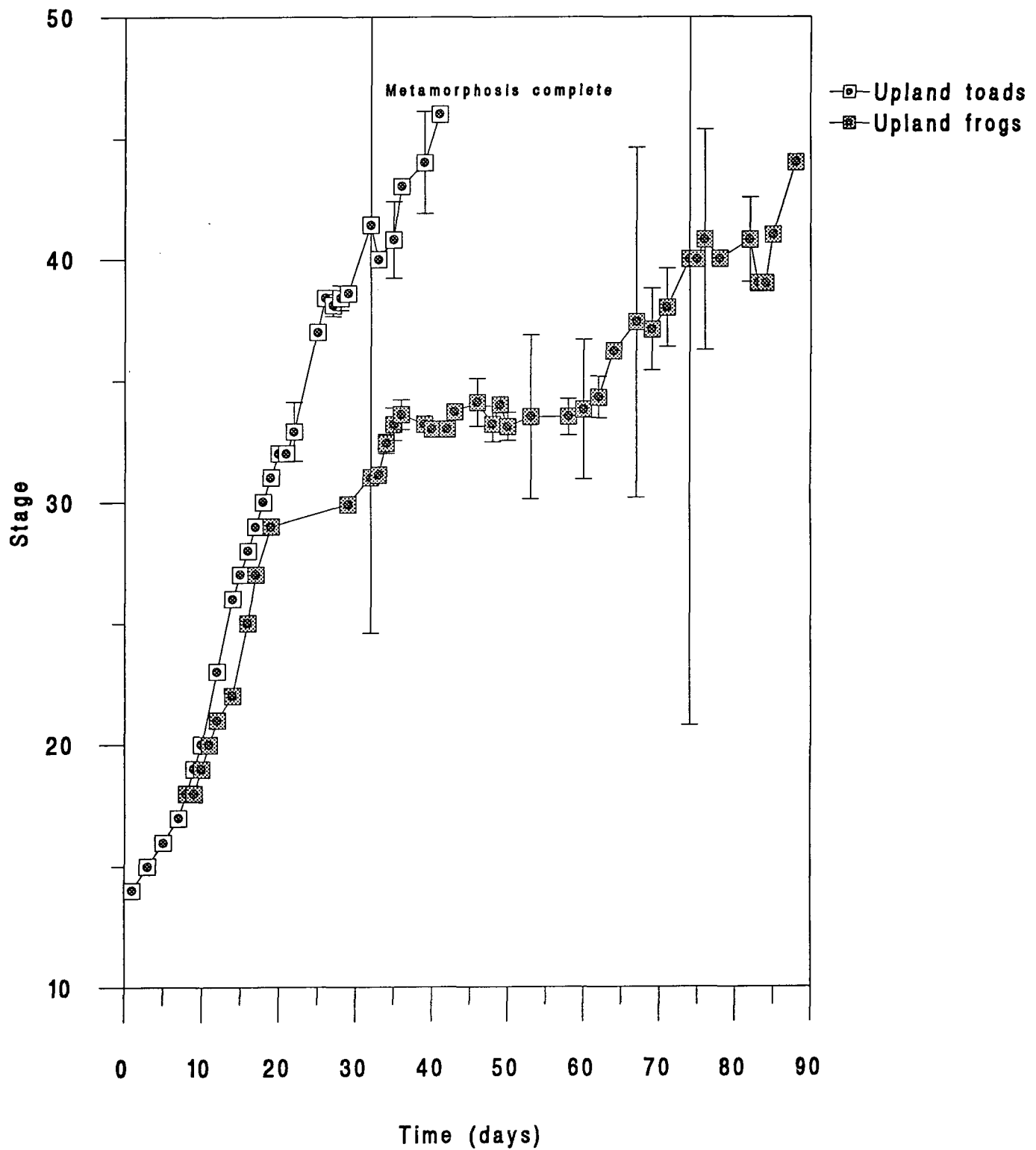


Table 5. Mean stage  $\pm$  standard error reached by lowland frogs and toads at different temperatures after 42 days. The starting stage was 31 in all cases. n is the number of tadpoles surviving to 42 days.

	Temperature ( $^{\circ}\text{C}$ )			
	15 $^{\circ}\text{C}$		25 $^{\circ}\text{C}$	
	n	mean $\pm$ SE	n	mean $\pm$ SE
Lowland frogs	26	41.5 $\pm$ .43	11	39.0 $\pm$ 0
Lowland toads	8	33.8 $\pm$ .17	5	38.6 $\pm$ 1.1

Table 6. compares the mean stage reached by lowland frogs and toads after 42 days using a t-test and gives the p-value that this calculation represents. It also shows which animal was more advanced at the time when the recordings were taken and by how many stages.

Table 6. A comparison between the stages reached by lowland frogs and toads at each temperature

Temperature ( $^{\circ}\text{C}$ )	Difference in mean stage	t-value	Degrees of freedom	p value
15 $^{\circ}\text{C}$	Frogs +7.7	16.65	32	<0.05
25 $^{\circ}\text{C}$	Frogs +0.4	0.36	14	>0.05

Figure 6. shows the development curves for lowland frogs and toads at 15 $^{\circ}\text{C}$ . Lowland frogs develop much faster than lowland toads and the difference of 7.7 stages after 42 days is highly significant as can be seen in table 4.

Figure 7. shows the development curves for lowland frogs and toads at 25 $^{\circ}\text{C}$ . The two populations display little difference in the mean stage reached and the t-value of 0.36 is not significant. The large standard error recorded for the lowland toad population after 67 days results from the fact that the population is very small by this time and thus any individuals in the



Figure 6. The Mean Development Stage of Lowland Frogs and Toads at 15 °C With Standard Error Bars Giving 95% Confidence Limits

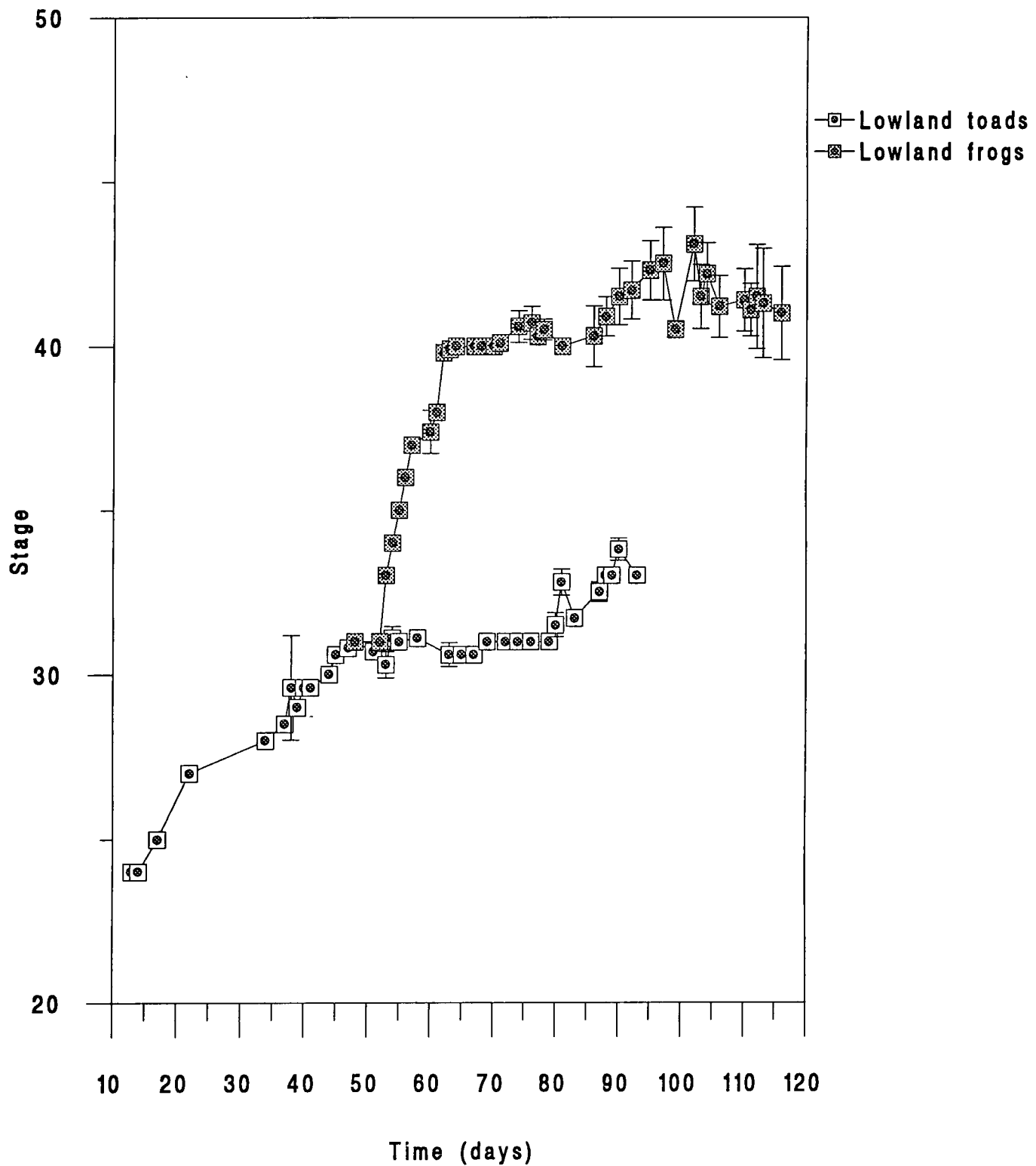
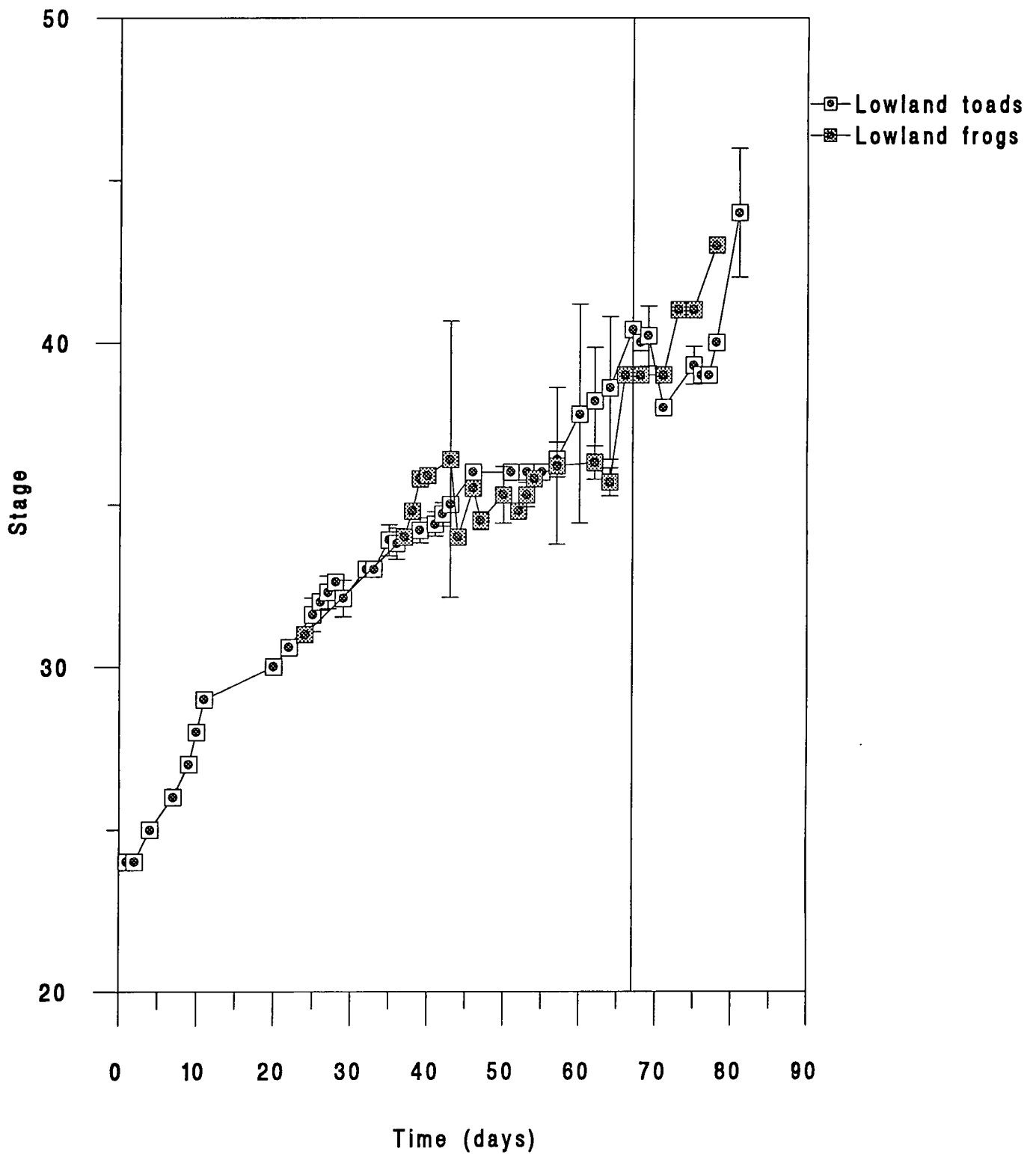


Figure 7. The Mean Development Stage of Lowland Frogs and Toads at 25°C With Standard Error Bars Giving 95% Confidence Limits



population that are several stages less developed than the majority cause a very large standard error to be produced.

In conclusion lowland frogs develop more rapidly than lowland toads at 15°C but at 25°C there is no difference between the development rates of these two populations because the development of lowland frogs does not increase as the temperature rises from 15°C to 25°C. The comparison between the two populations at 15°C is therefore likely to be most representative and at this temperature lowland frogs are the faster developers.

### 3.1.3. A Comparison Between the Development Rates of Upland and Lowland Frogs

Table 7. shows the mean stages reached by upland and lowland frogs developing at a series of different constant temperatures after 44 days. The mean starting stage was 31 for all the cultures. However, the only overlap in development stages occurred at 25°C and this is therefore the only temperature at which comparisons can be made.

Table 7. Mean stage  $\pm$  standard error reached by upland and lowland frogs at different temperatures after 44 days. The starting stage was 31 in all cases. n is the number of tadpoles surviving to 44 days.

	Temperature (°C)	
	25°C	
	n	mean $\pm$ SE
Upland frogs	4	40.8 $\pm$ 2.28
Lowland frogs	9	39.0 $\pm$ 0

Table 8. compares the mean stage reached by upland and lowland frogs after 44 days using a t-test and gives the p-value that this calculation represents. It also shows which animal was more advanced at the time when the recordings were taken and by how many stages.

Table 8. A comparison between the stages reached by upland and lowland frogs at each temperature

Temperature (°C)	Difference in mean stage	t-value	Degrees of freedom	p value
25°C	Upland frogs +1.8	0.79	11	>0.05

Figure 8. shows the development curves of upland and lowland frogs at 25°C. This was the only incubation temperature at which the upland and lowland frogs completed the same development stages because the upland frogs were much less advanced (stage 18) than the lowland frogs (stage 31) when collected from the wild.

No obvious differences are apparent and this is supported by the t-value of 0.79 which indicates that after 44 days there is no significant difference between the mean development stages of the two populations.

To conclude, there is no evidence to indicate that there is any difference between the development rates of upland and lowland frogs.

#### 3.1.4. A Comparison Between the Development Rates of Upland and Lowland Toads

Table 9. shows the mean stages reached by upland and lowland toads developing at a series of different constant temperatures after 28 days. The mean starting stage was 24 for all the cultures and it can be seen that the development rate of lowland frogs decreased from 15°C to 25°C but increased over this range for lowland toads.

Figure 8. The Mean Development Stage of Upland and Lowland Frogs at 25 °C With Standard Error Bars Giving 95% Confidence Limits

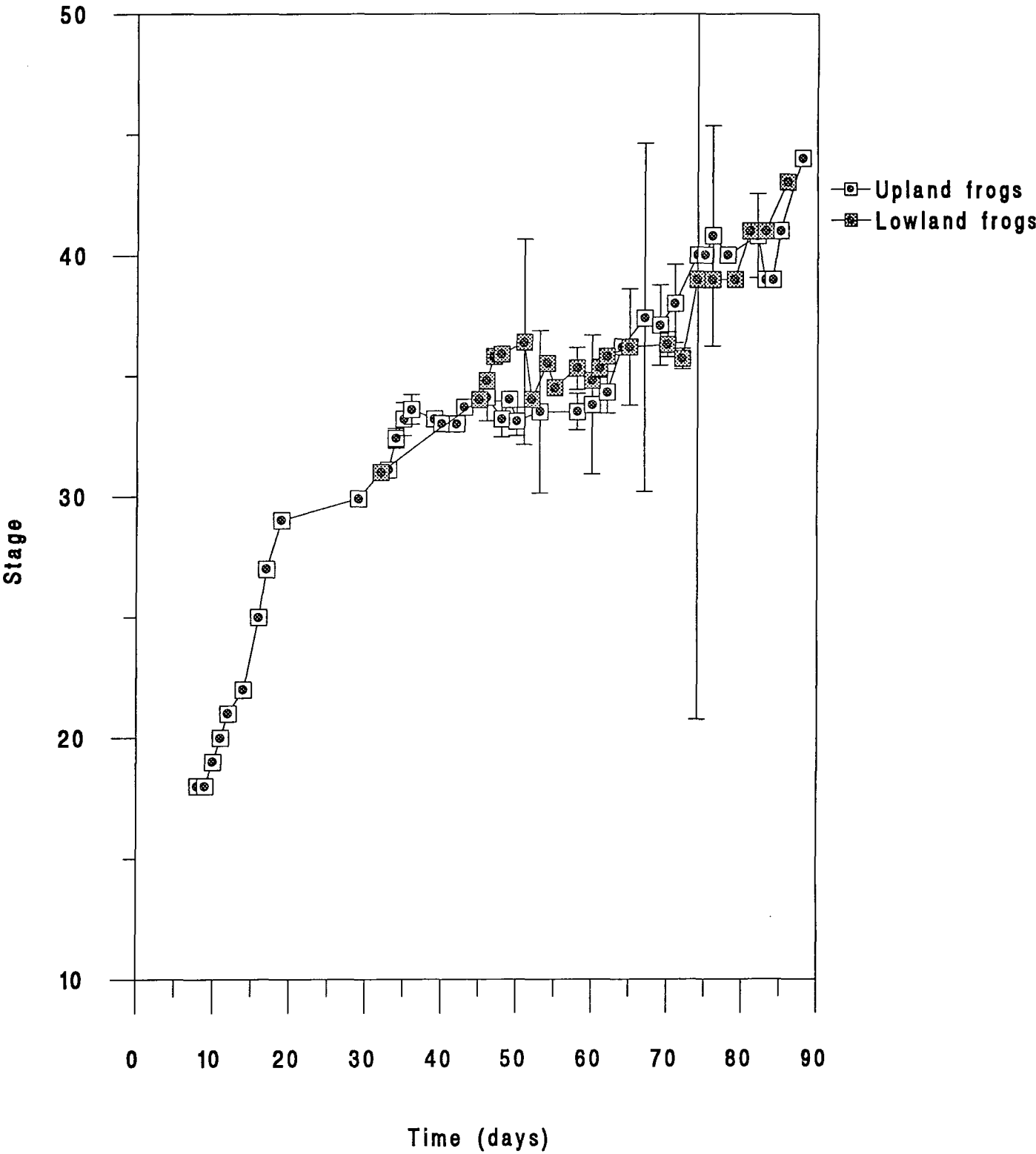


Table 9. Mean stage  $\pm$  standard error reached by upland and lowland toads at different temperatures after 28 days. The starting stage was 24 in all cases. n is the number of tadpoles surviving to 28 days.

	Temperature ( $^{\circ}\text{C}$ )									
	5 $^{\circ}\text{C}$		8 $^{\circ}\text{C}$		10 $^{\circ}\text{C}$		15 $^{\circ}\text{C}$		25 $^{\circ}\text{C}$	
	n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE
Upland toad	-		21	28.0 $\pm$ 0	16	28.0 $\pm$ 0	42	31.0 $\pm$ 0	15	46.0 $\pm$ 0
Lowland toad	-		53	28.0 $\pm$ 0	46	28.7 $\pm$ .07	40	29.6 $\pm$ .10	16	32.6 $\pm$ .13

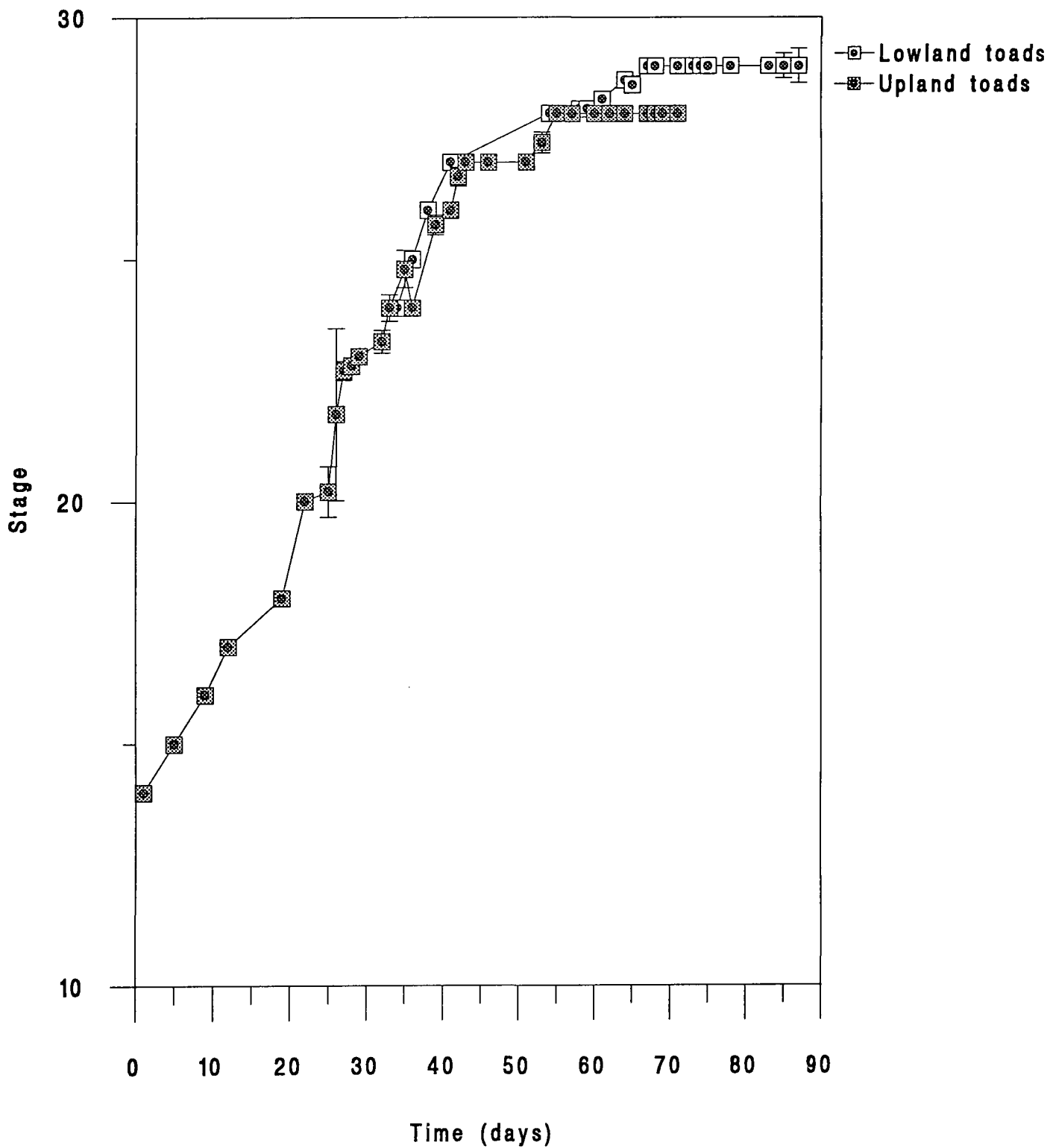
Table 10. compares the mean stage reached by upland and lowland toads after 27 days using a t-test and gives the p-value that this calculation represents. It also shows which animal was more advanced at the time when the recordings were taken and by how many stages.

Table 10. A comparison between the stages reached by upland and lowland toads at each temperature

Temperature ( $^{\circ}\text{C}$ )	Difference in mean stage	t-value	Degrees of freedom	p value
5 $^{\circ}\text{C}$		-		
8 $^{\circ}\text{C}$	0	0	72	>0.05
10 $^{\circ}\text{C}$	Lowland toads +0.7	10.00	60	<0.05
15 $^{\circ}\text{C}$	Upland toad +1.4	14.00	80	<0.05
25 $^{\circ}\text{C}$	Upland toad +13.4	103.08	29	<0.05

Figure 9. shows the development curves of upland and lowland toads at 8 $^{\circ}\text{C}$ . These curves are very similar with no significant difference in the mean development stage after 28 days.

Figure 9. The Mean Development Stage of Upland and Lowland Toads at 8°C With Standard Error Bars Giving 95% Confidence Limits



However, by day 60 the lowland toads have developed slightly faster and are approximately 1 stage more advanced than the upland toad population.

Figure 10. shows the development curves of upland and lowland toads at 10°C with lowland toads being slightly more advanced by day 50. Table 8. also confirms this pattern with lowland toads being 0.3 of a stage significantly more advanced after 28 days.

Figure 11. shows the development curves of upland and lowland toads at 15°C. The upland toads initially develop more rapidly than the lowland population and the t-value of 14.00 after 28 days supports this i.e. at this point the upland toads are 1.4 stages significantly more advanced than the lowland. However, Figure 11. shows that by day 80 there is no significant difference between the mean stage reached by these two populations.

Figure 12. shows the development curves of upland and lowland toads at 25°C At this temperature the development rate of upland toads substantially exceeds that of their lowland counterparts and they had completed metamorphosis when the lowland toads were still at approximately stage 32. The t-value of 103.08 from table 8 indicates that this difference is highly significant after 28 days.

To conclude, upland toads developed more rapidly than lowland toads at 15°C and 25°C.

#### 3.1.5. Summary of the Effect of Temperature on the Development Rates of Upland and Lowland Frogs and Toads

Figure 13. summarises the development of upland frogs and toads at different temperatures. Upland frogs developed faster than upland toads at 5°C, 8°C and 10°C but the development of upland toads was the most rapid at 15°C and 25°C. This figure shows that upland toads display a more marked response to increasing temperature than upland frogs.

Figure 14. summarises the development of lowland frogs and toads at different temperatures. Lowland frogs developed faster at 15°C but at 25°C their development was equal to that of lowland toads.

Upland and lowland frogs only completed the same stages at 25°C and at this temperature there was no difference between their rate of development.



Figure 10. The Mean Development Stage of Upland and Lowland Toads at 10 °c With Standard Error Bars Giving 95% Confidence Limits

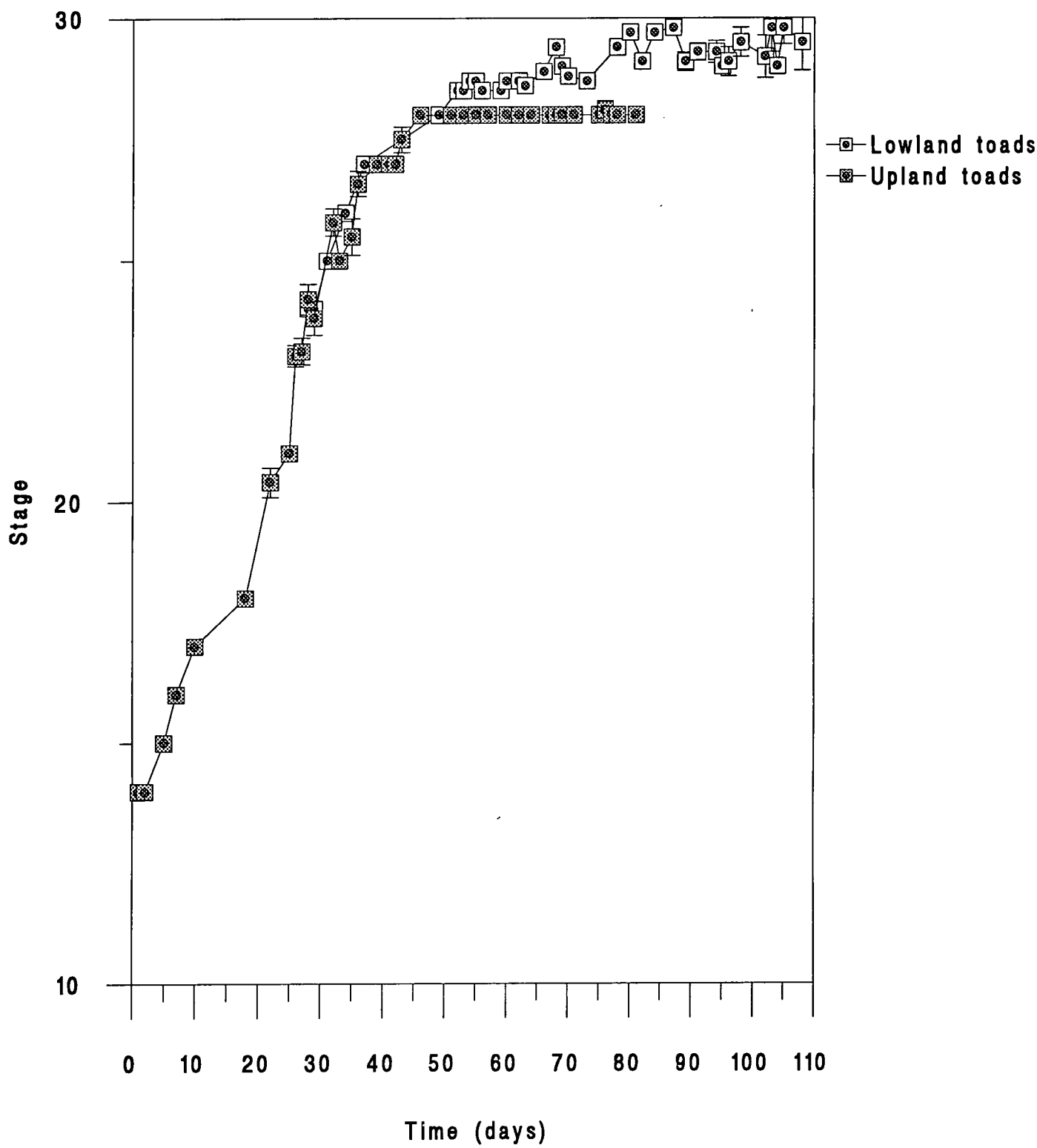
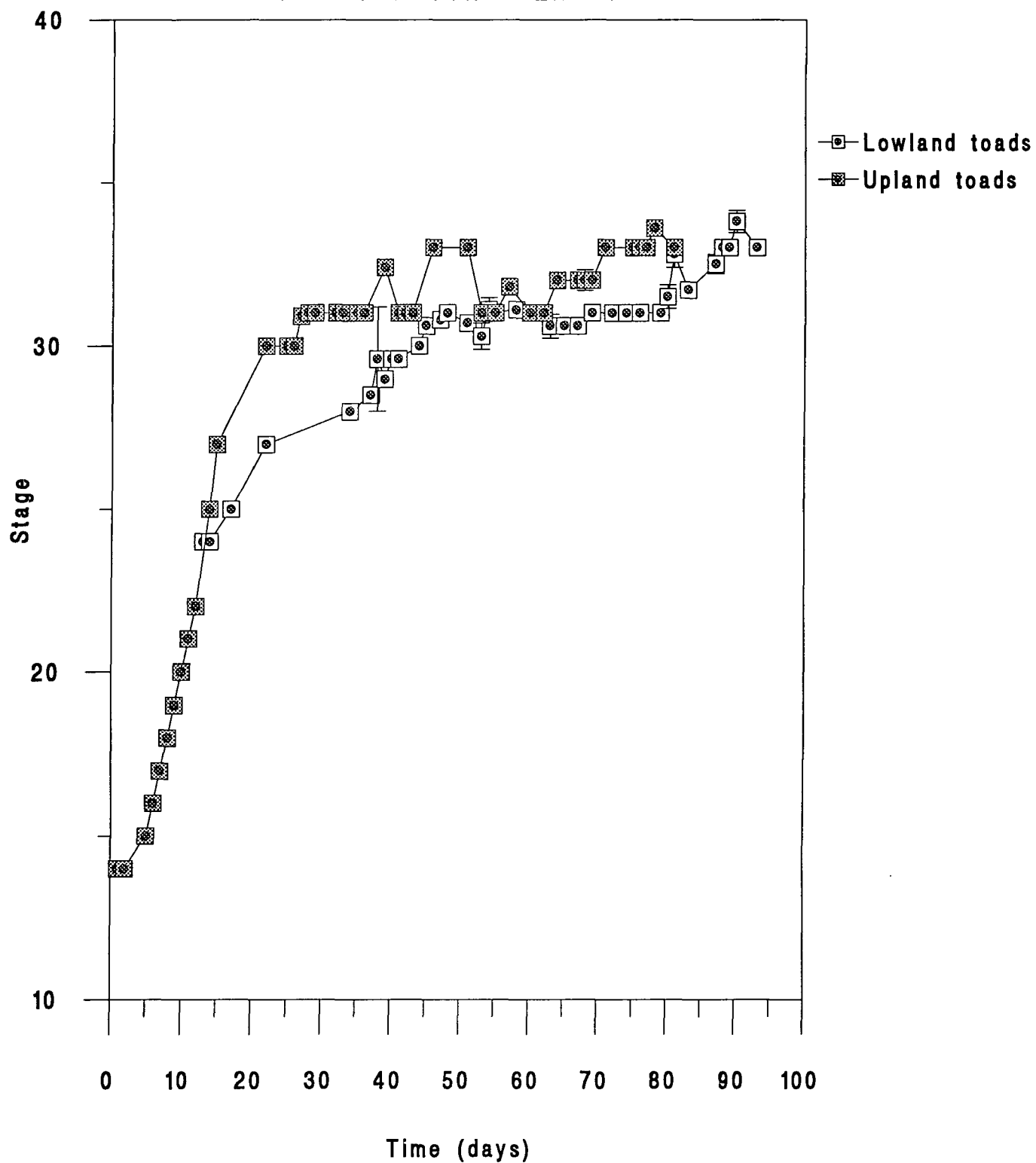


Figure 11. The Mean Development Stage of Upland and Lowland Toads at 15 °C With Standard Error Bars Giving 95% Confidence Limits



**Figure 12. The Mean Development Stage of Upland and Lowland Toads at 25 °C With Standard Error Bars Giving 95% Confidence Limits**

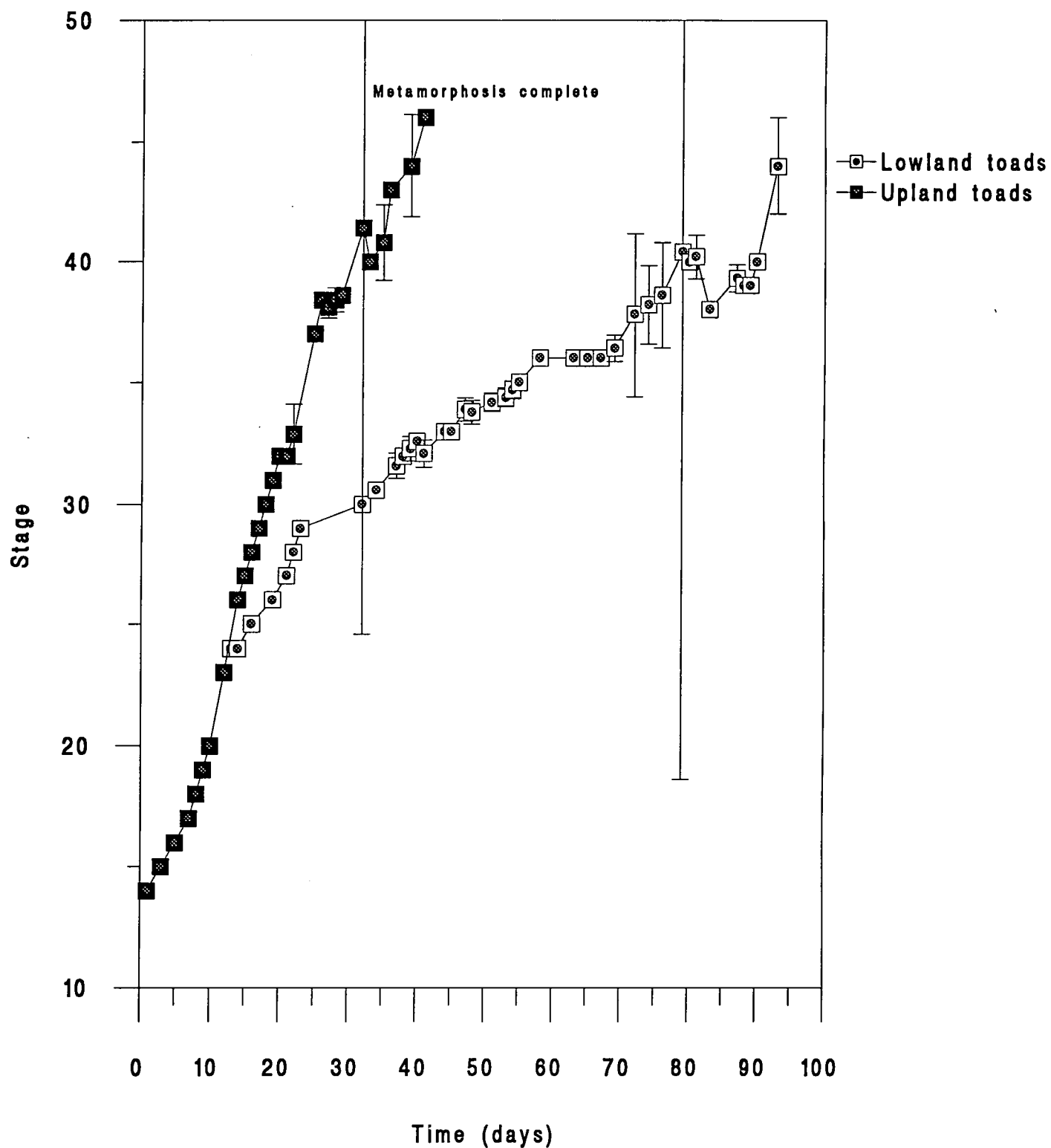


Figure 13. The Mean Stage Reached By Upland Frogs and Toads After 27 Days At Different Temperatures

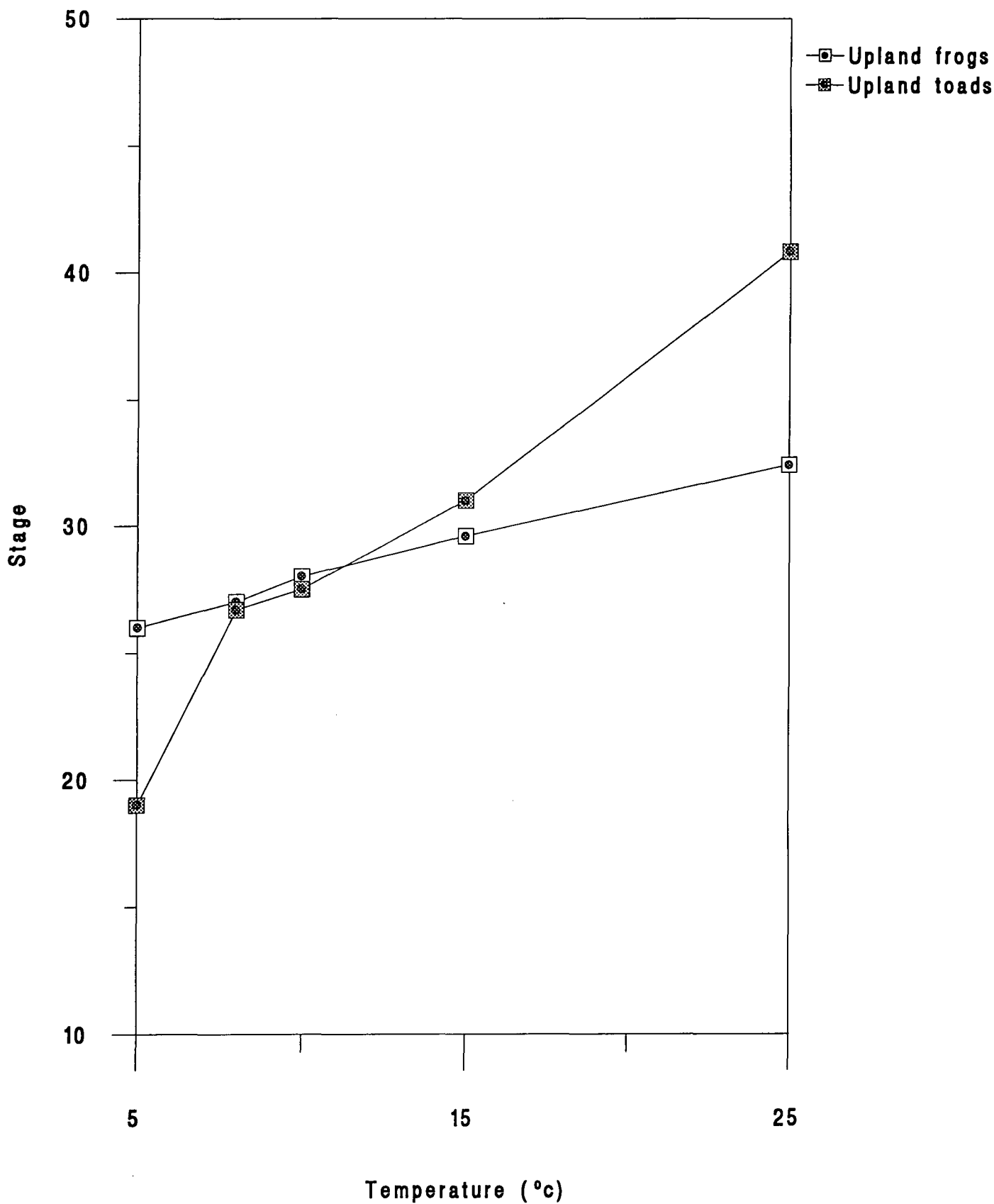


Figure 14. The Mean Stage Reached By Lowland Frogs and Toads After 42 Days at Different Temperatures

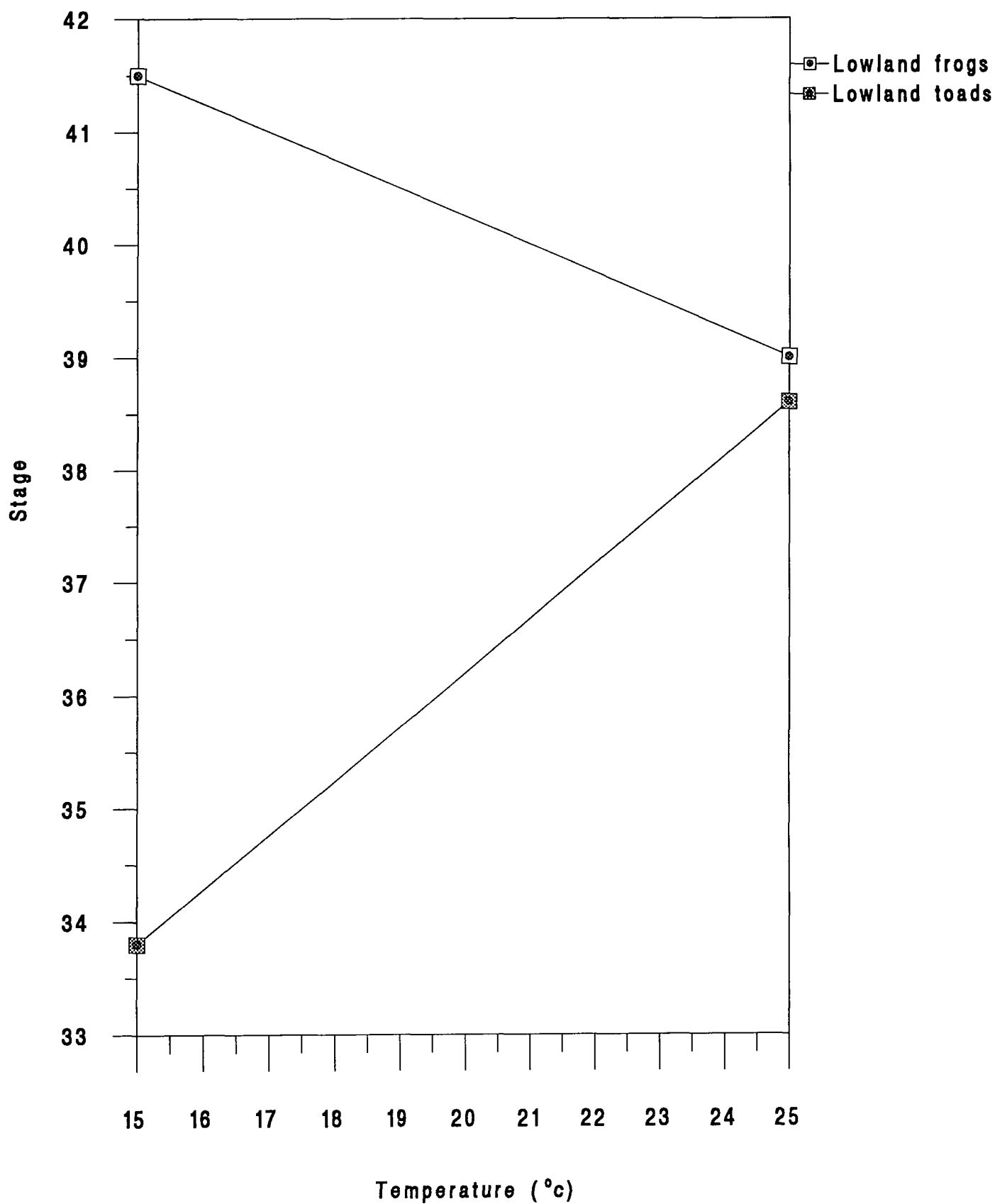


Figure 15. summarises the development of upland and lowland toads at different temperatures. Lowland toads developed more rapidly than upland toads at 8°C and 10°C but at 15°C and 25°C upland toads are the quicker developers.

Figure 16. shows the development of upland frogs at 5°C, 8°C, 10°C, 15°C and 25°C. As the temperature increases the mean development stage reached in a given time also increases. In addition, as the temperature increases the amount of variation around each mean also increases because more stages have been completed.

Figure 17. shows the development of upland toads at 5°C, 8°C, 10°C, 15°C and 25°C. Again, increasing temperature increases the rate of development and also the degree of variation.

Figure 18. shows the development of lowland frogs at 5°C, 8°C, 10°C, 15°C and 25°C. Development is initially fastest at 15°C but by approximately day 67 the lowland frogs cultured at 25°C have caught and overtaken the mean development stage of those cultured at 15°C.

Figure 19. shows the development of lowland toads at 5°C, 8°C, 10°C, 15°C and 25°C. For this culture increasing temperature resulted in the more rapid development of tadpoles and an increase in variation.

### 3.2 Mortality

The full mortality data is displayed in Appendix C. The following tables display summaries of the relevant sections of this information.

#### 3.2.1. The Effect of Temperature on Frog and Toad Mortality

Figure 20. shows the mortality curves for upland frogs at different temperatures. There is a general trend for a more rapid decrease in population size as the temperature increases from 5°C to 25°C.

Figure 21. shows the mortality curves for upland toads at different temperatures. Here the most rapid population decline occurs at 5°C, 8°C and 25°C.

Figure 22. shows the mortality curves for lowland frogs at different temperatures. The highest mortality occurs at 5°C and 25°C. Unusually, the population at 8°C suffered no mortality.

Figure 23. shows the mortality curves for lowland toads at different temperatures. The most rapid decrease in population numbers occurred at 5°C, 8°C and 25°C.

Figure 15. The Mean Stage Reached By Upland and Lowland Toads After 28 Days at Different Temperatures

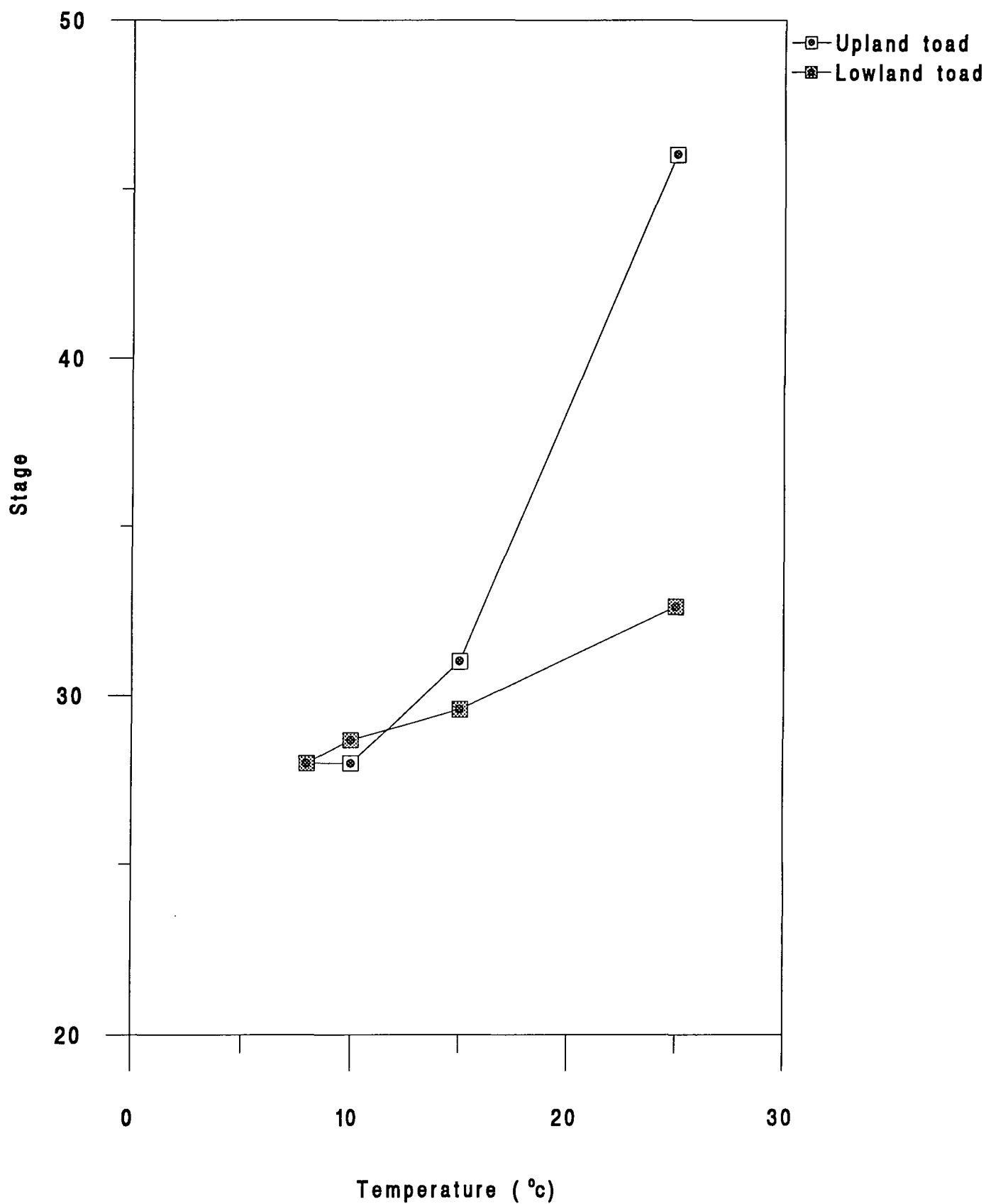


Figure 16. The effect of temperature on the development rate of upland frogs

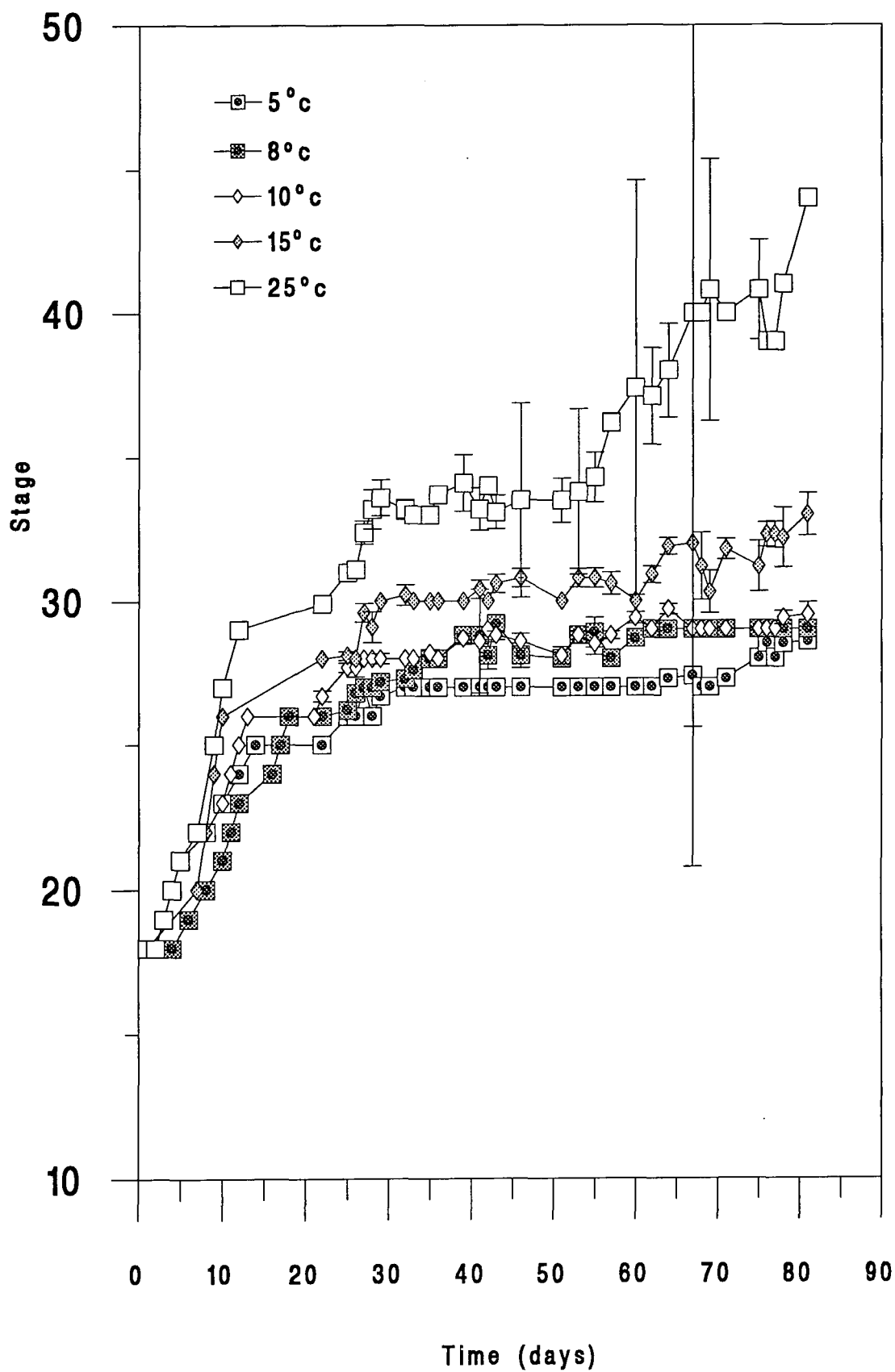




Figure 17. The effect of temperature on the development of upland toads

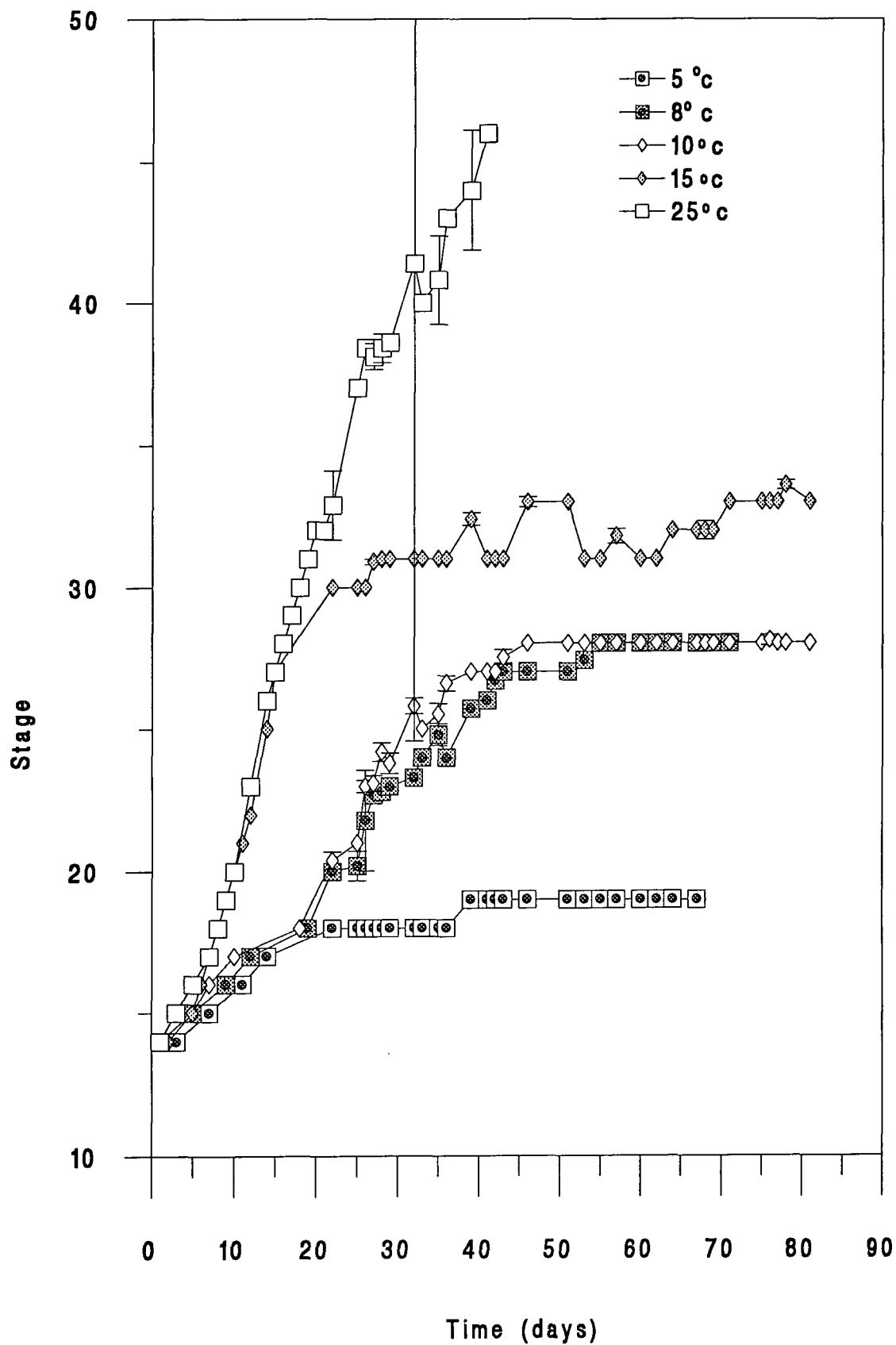


Figure 18. The effect of temperature on the development of lowland frogs

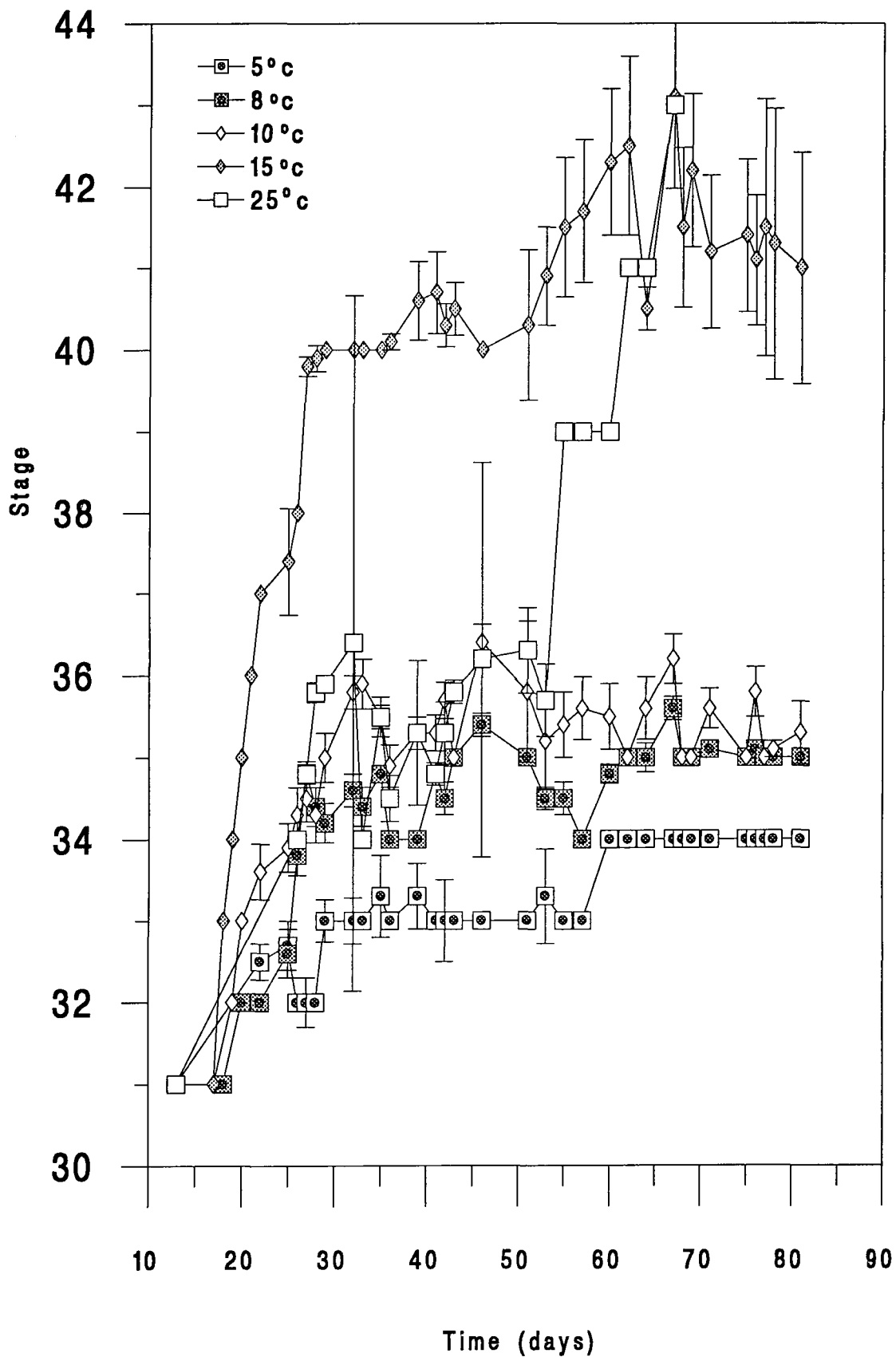


Figure 19. The effect of temperature on the development of lowland toads

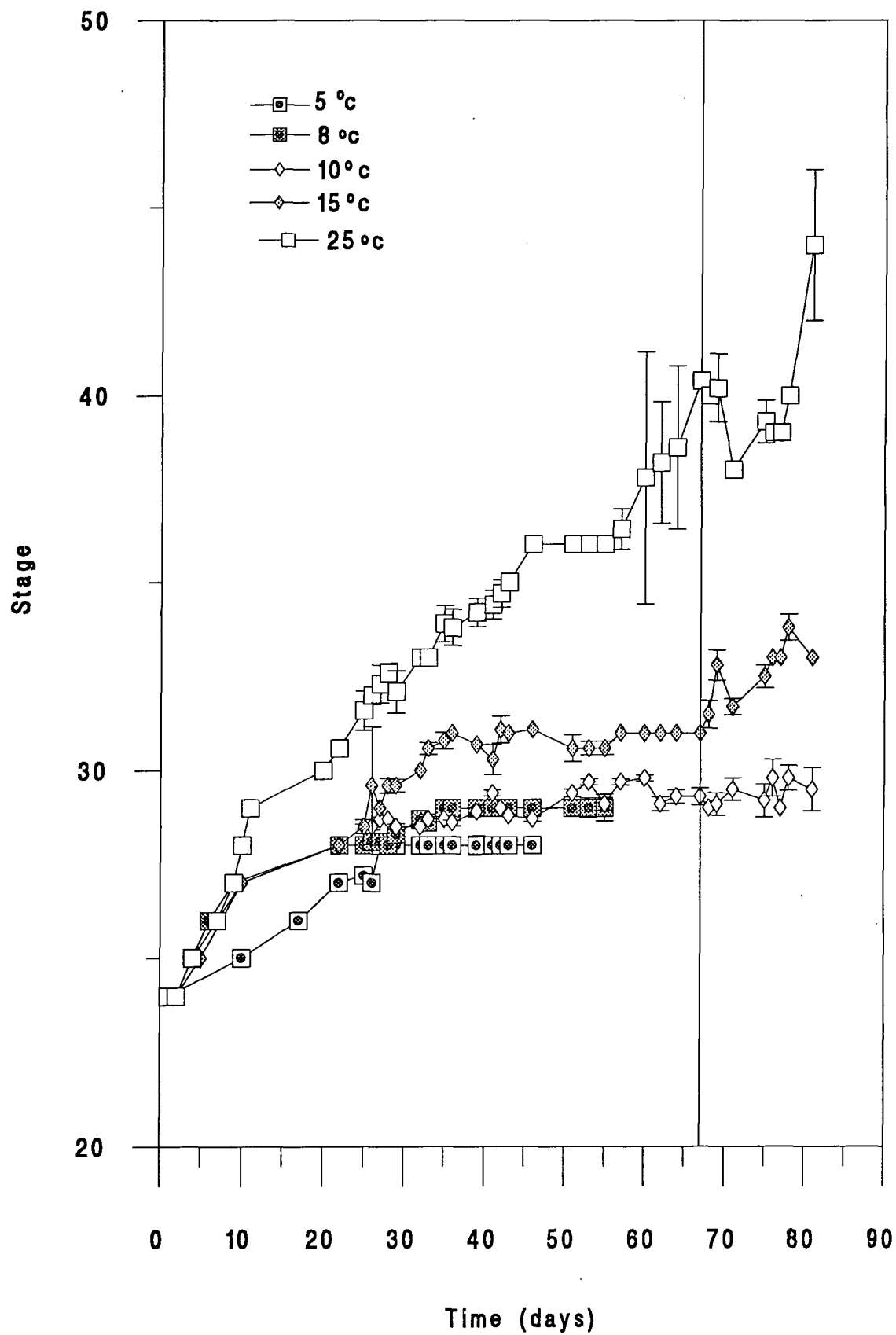


Figure 20. The effect of temperature on upland frog mortality

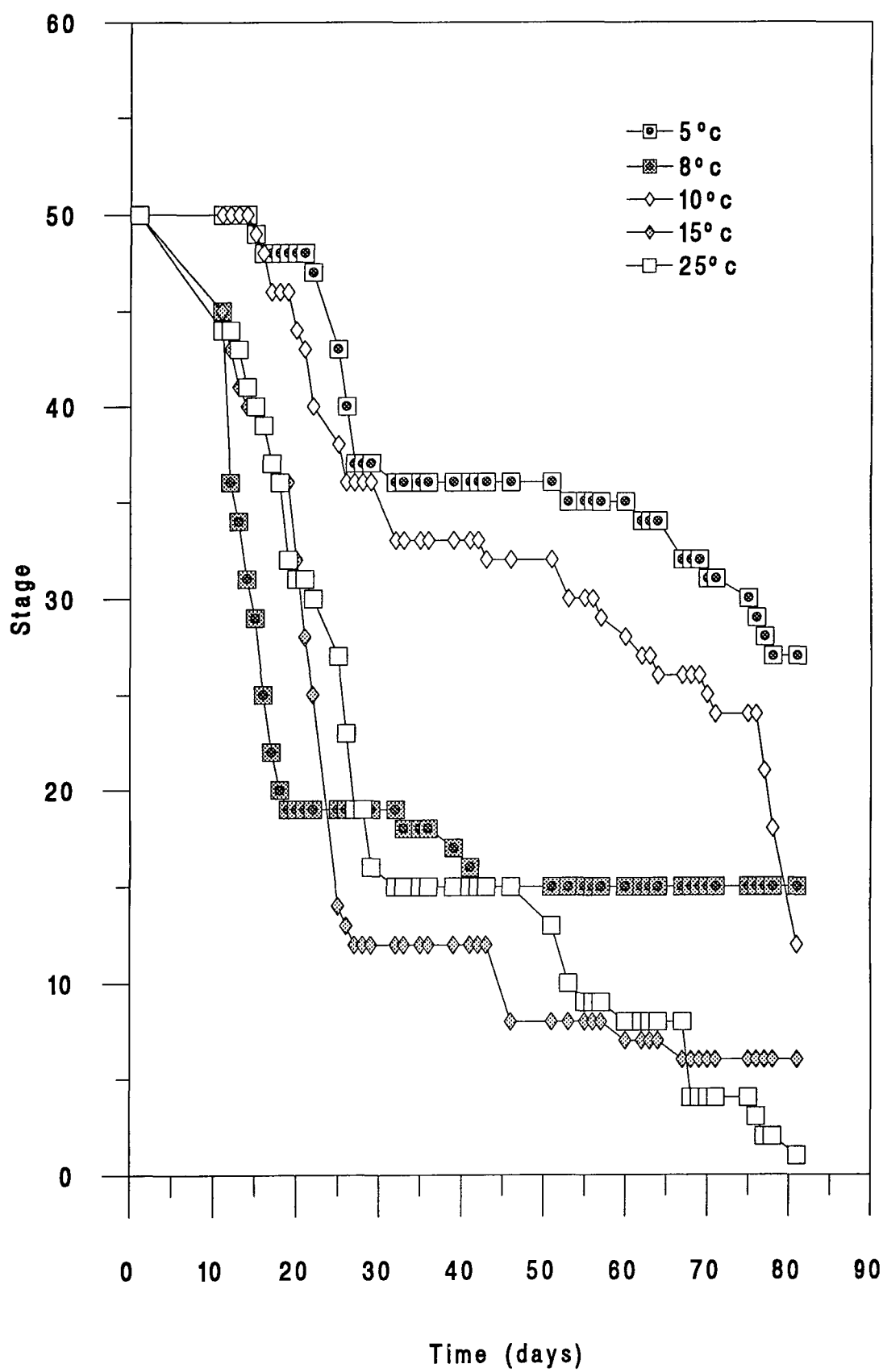


Figure 21. The effect of temperature on upland toad mortality

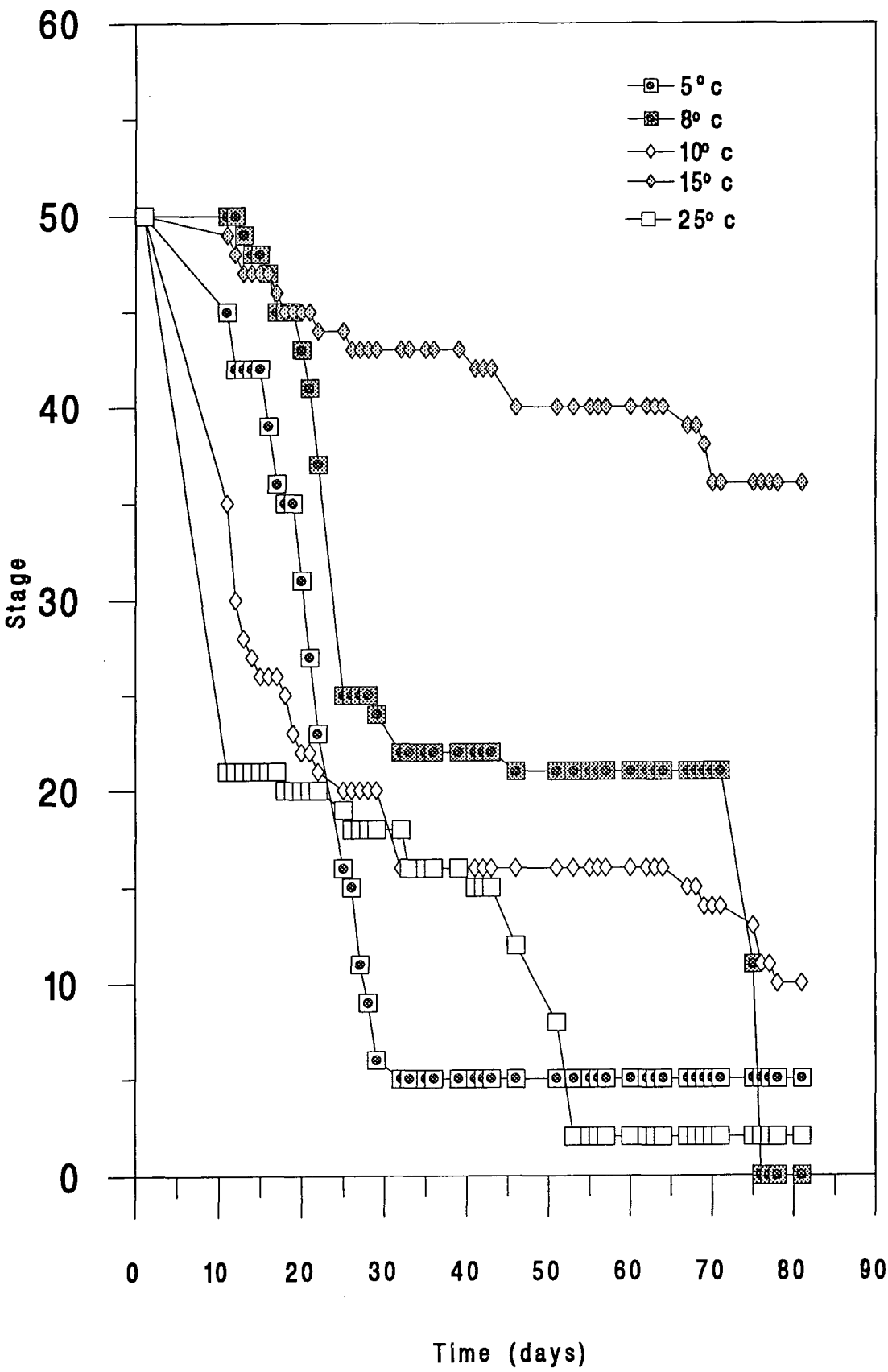


Figure 22. The effect of temperature on lowland frog mortality

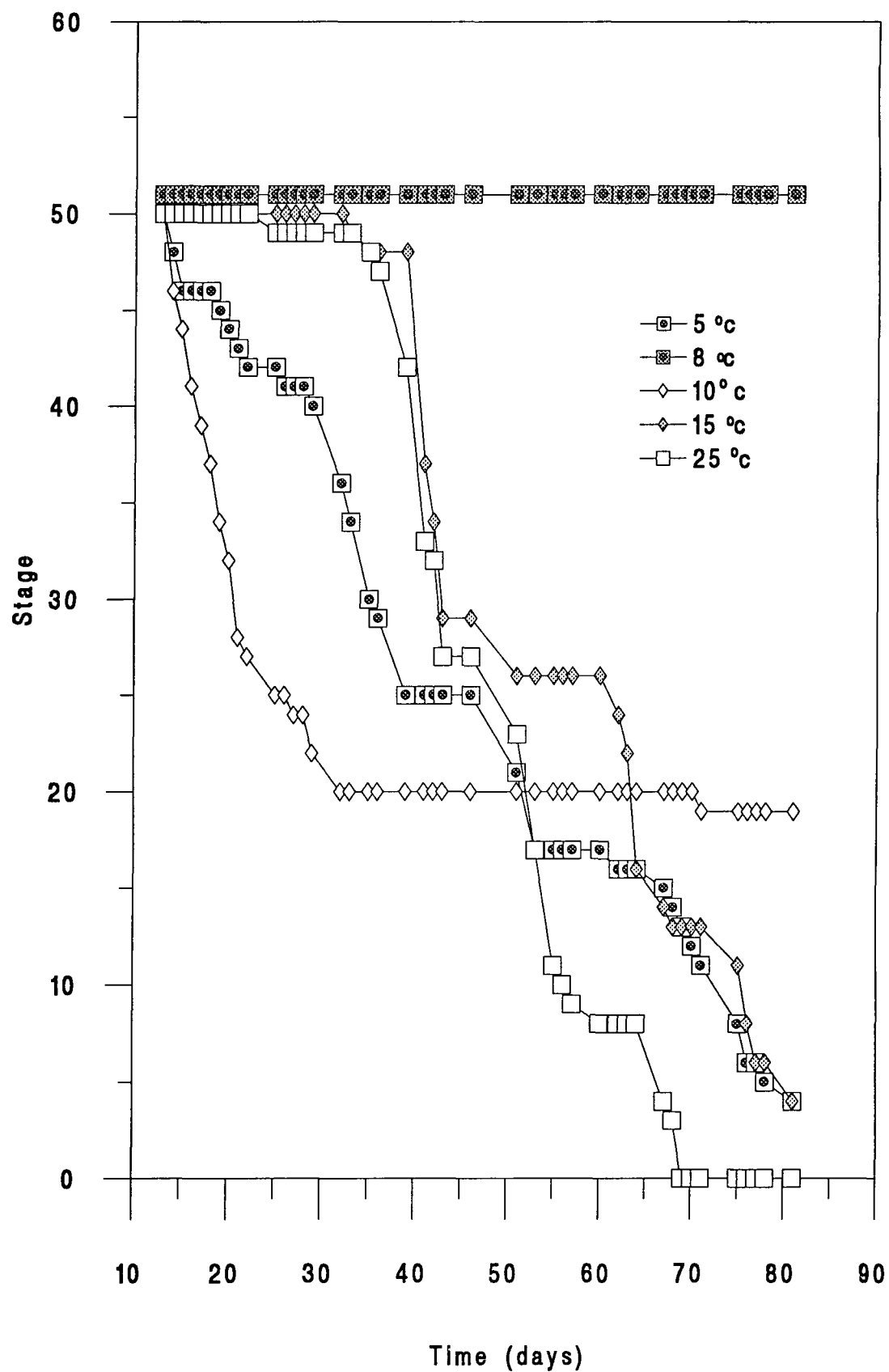
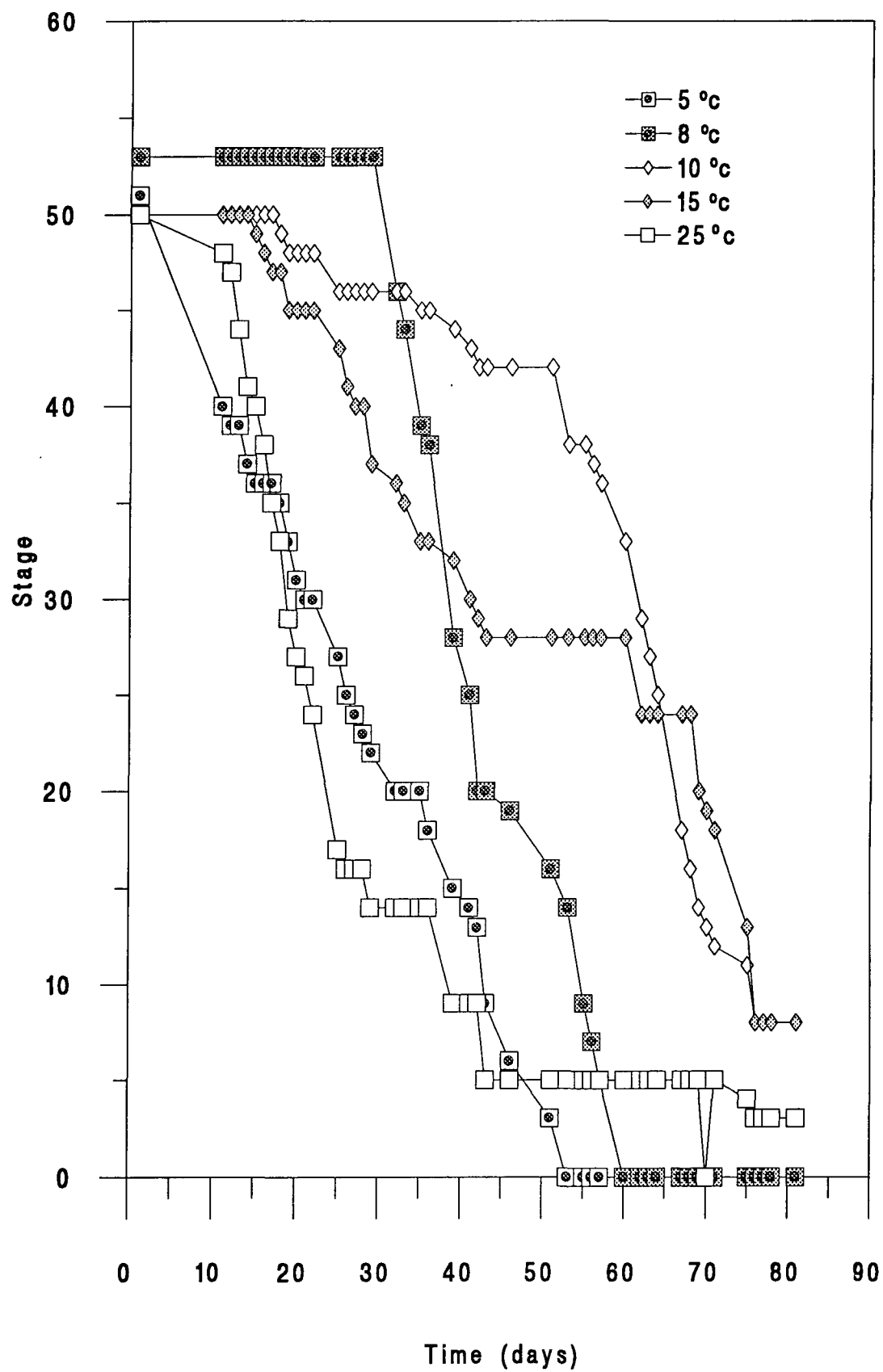


Figure 23. The effect of temperature on the mortality of lowland toads



All the populations except lowland frogs at 8°C display similar patterns of mortality with a rapid initial decrease in population size which then stabilises after which the rate of decrease is much slower. The rapid initial decline could be caused either by overcrowding leading to competition for food and space or by the death of the weaker individuals in the population.

These mortality results are summarised in the following table which shows the percentage of each population still alive after 81 days i.e. at the end of the investigation.

Table 11. The percentage survival of upland frogs, upland toads, lowland frogs and lowland toads after 81 days at different temperatures.

Population	Temperatures (°C)				
	5°C	8°C	10°C	15°C	25°C
Upland frogs	54	15	24	12	2
Upland toads	10	0	20	72	4
Lowland frogs	8	100	38	8	0
Lowland toads	0	0	15	16	6

Few clear patterns are discernible regarding the effect of temperature on survivorship. All four populations exhibit low survival rates at 25°C and, except for the upland frogs, at 5°C too. The intermediate temperatures of 8°C, 10°C and 15°C appear most favourable for tadpole survival.

### 3.2.2. A Comparison of Survivorship Curves Using the Logrank Statistic

The survival of upland and lowland frogs and toads was then compared at each culture temperature using the logrank statistic. Table 12. shows the log rank statistic at different temperatures for the four combinations of populations being studied. A value of 3.84 or greater indicates that the survivorship curves are significantly different and these figures have been



underlined. Under such circumstances the population which is the more advanced of the two under comparison has been written under the Chi-squared value.

Table 12. The log rank statistic for combinations of upland and lowland frogs and toads at different temperatures.

Population	Temperatures (°C)									
	5°C	p	8°C	p	10°C	p	15°C	p	25°C	p
Upland frogs and toads	<u>42.6</u> Toad	<0.05	2.33	>0.05	<u>6.95</u> Toad	<0.05	<u>49.60</u> Frog	<0.05	<u>8.02</u> Toad	<0.05
Lowland frogs and toads	<u>16.17</u> Toad	<0.05	<u>129.79</u> Toad	<0.05	0.026	>0.05	2.67	>0.05	<u>11.99</u> Toad	<0.05
Upland and lowland frogs	<u>24.86</u> Lowland	<0.05	<u>61.01</u> Upland	<0.05	0.083	>0.05	<u>11.34</u> Upland	<0.05	0.19	>0.05
Lowland and upland toads	0.27	>0.05	2.68	>0.05	3.14	>0.05	<u>29.30</u> Lowland	<0.05	0.76	>0.05

### 3.2.2.1. A Comparison of The Mortality of Upland Frogs and Toads at Different Temperatures

Figure 24. shows the mortality of upland frogs and toads at 5°C. Figure 25 shows the mortality of upland frogs and toads at 8°C. Figure 26 shows the mortality of upland frogs and toads at 10°C. Figure 27 shows the mortality of upland frogs and toads at 15°C. Figure 28 shows the mortality of upland frogs and toads at 25°C. At all these temperatures the populations decline quickly at first but this rate of decline slows down.

Figure 24. The Survival of Upland Frogs and Toads at 5 °c With Time

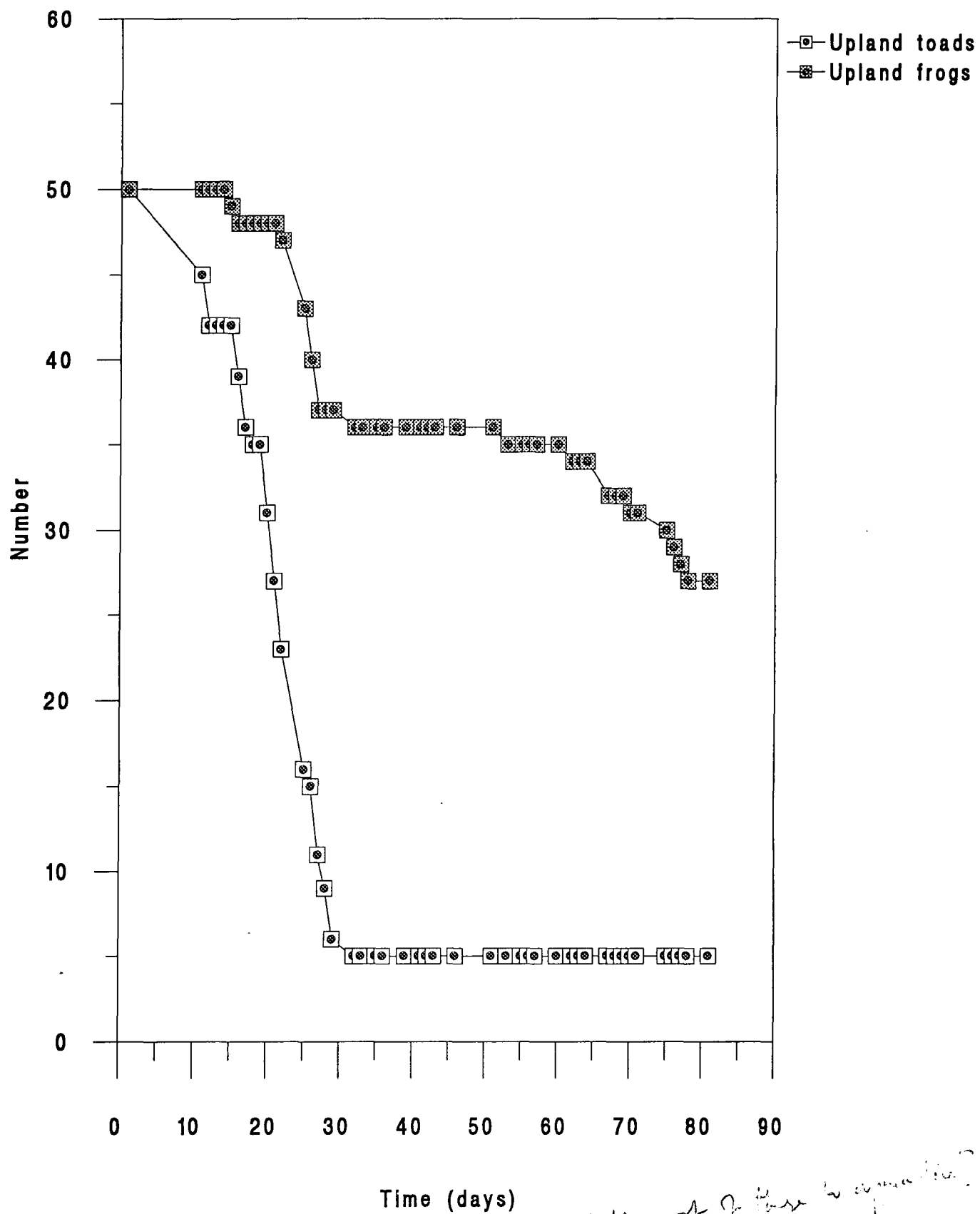


Figure 25. The Survival of Upland Frog and Toad Tadpoles at 8°C With Time

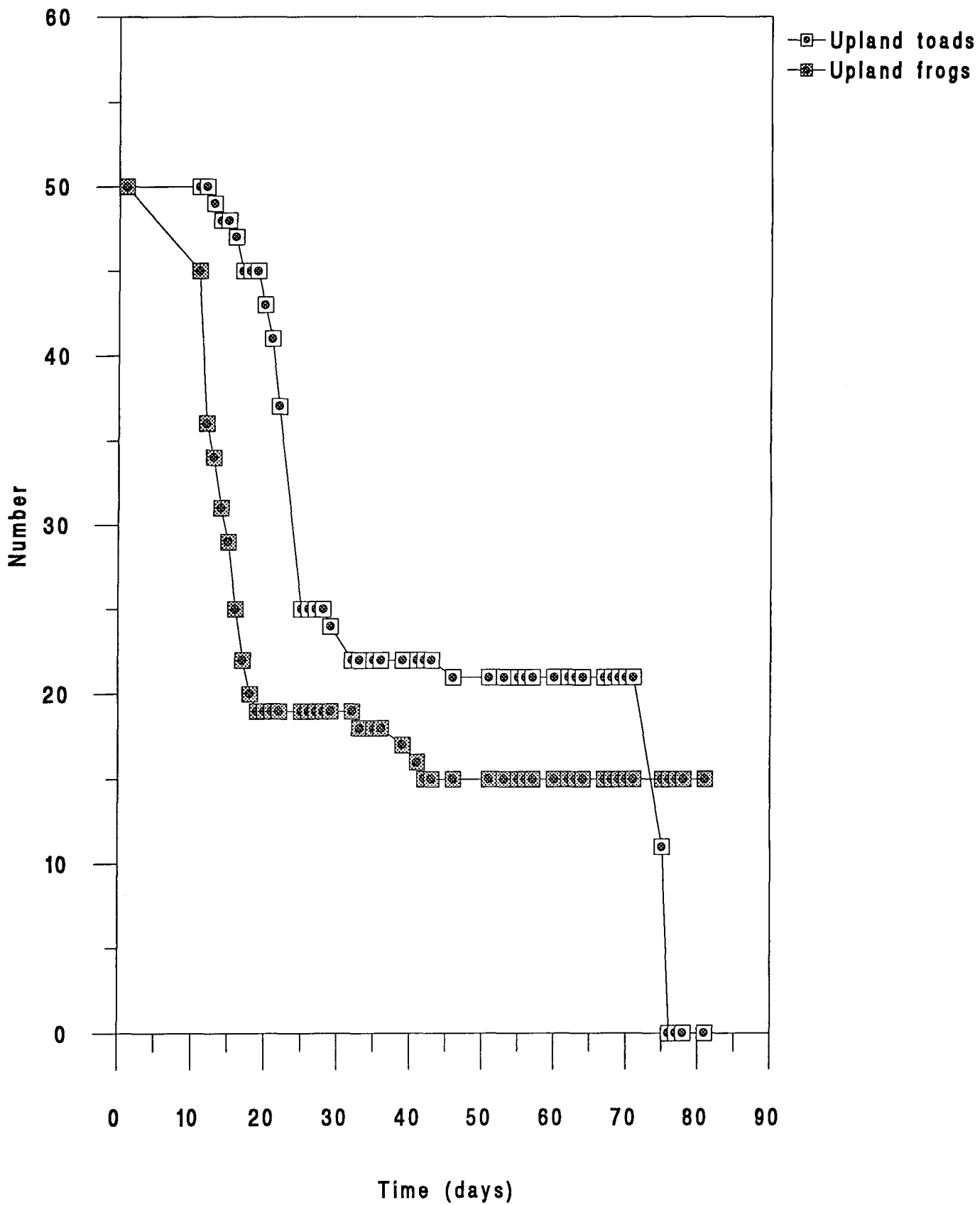


Figure 26. The Survival of Upland Frogs and Toads at 10°C With Time

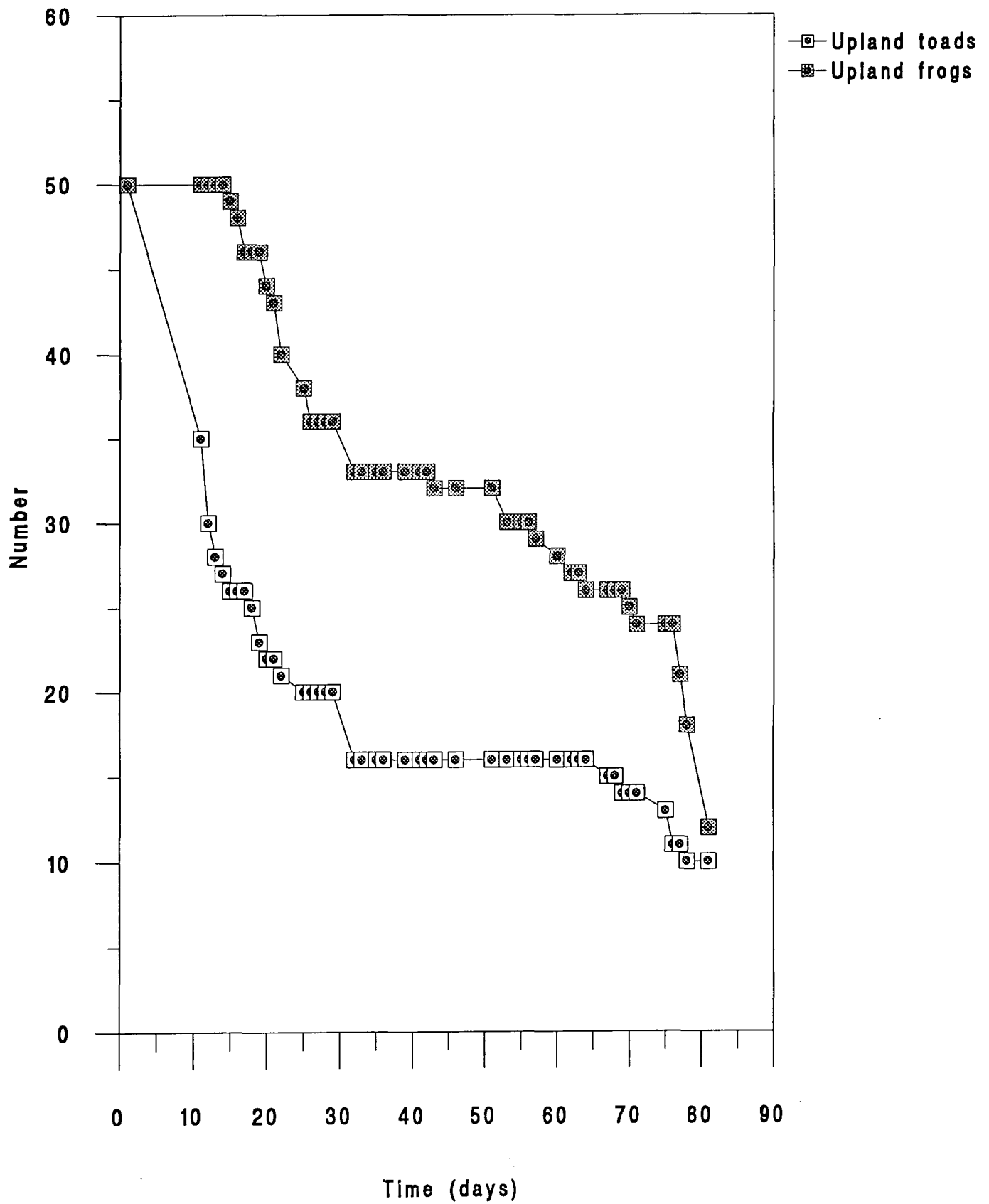


Figure 27. The Survival of Upland Frogs and Toads at 15°c With Time

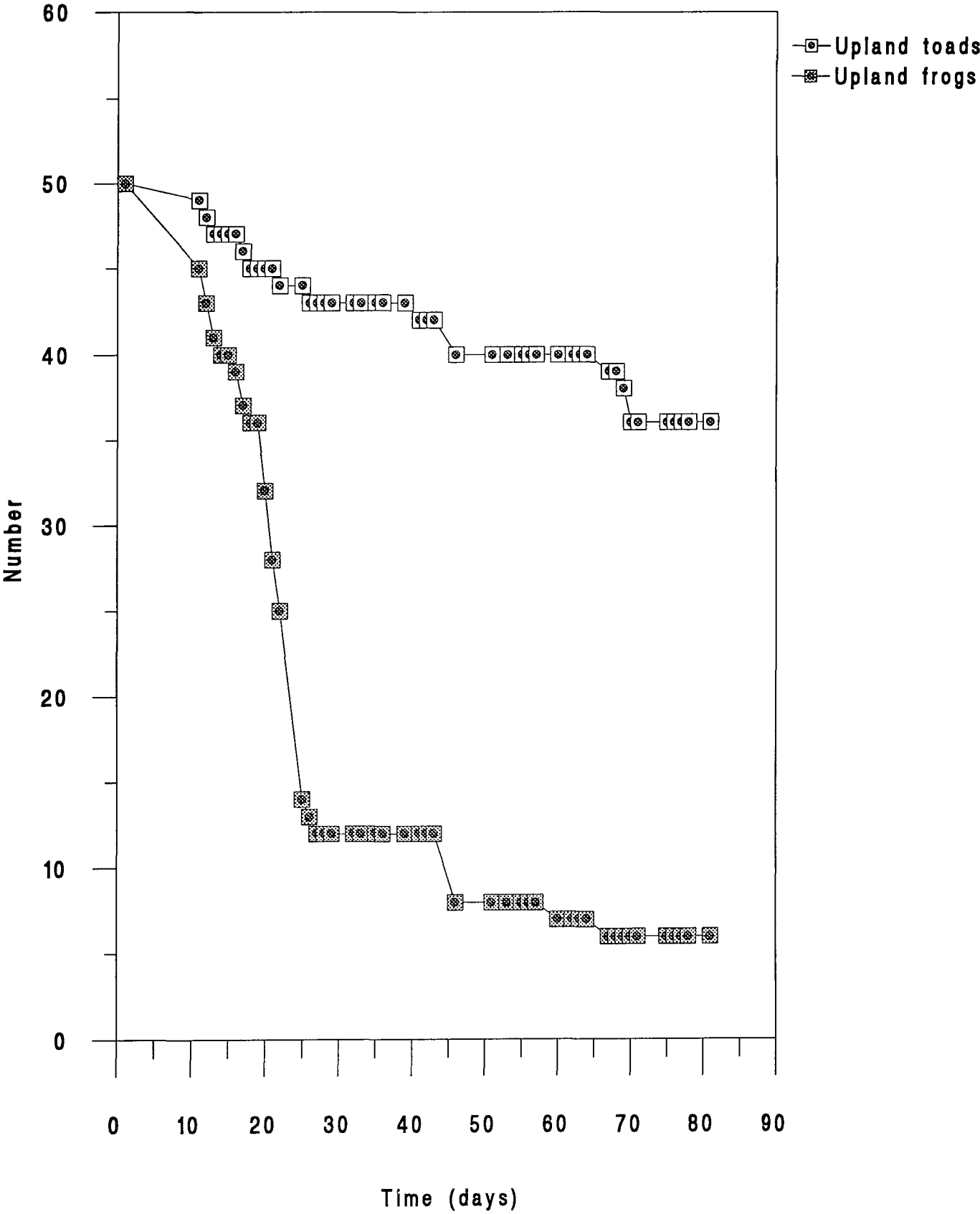
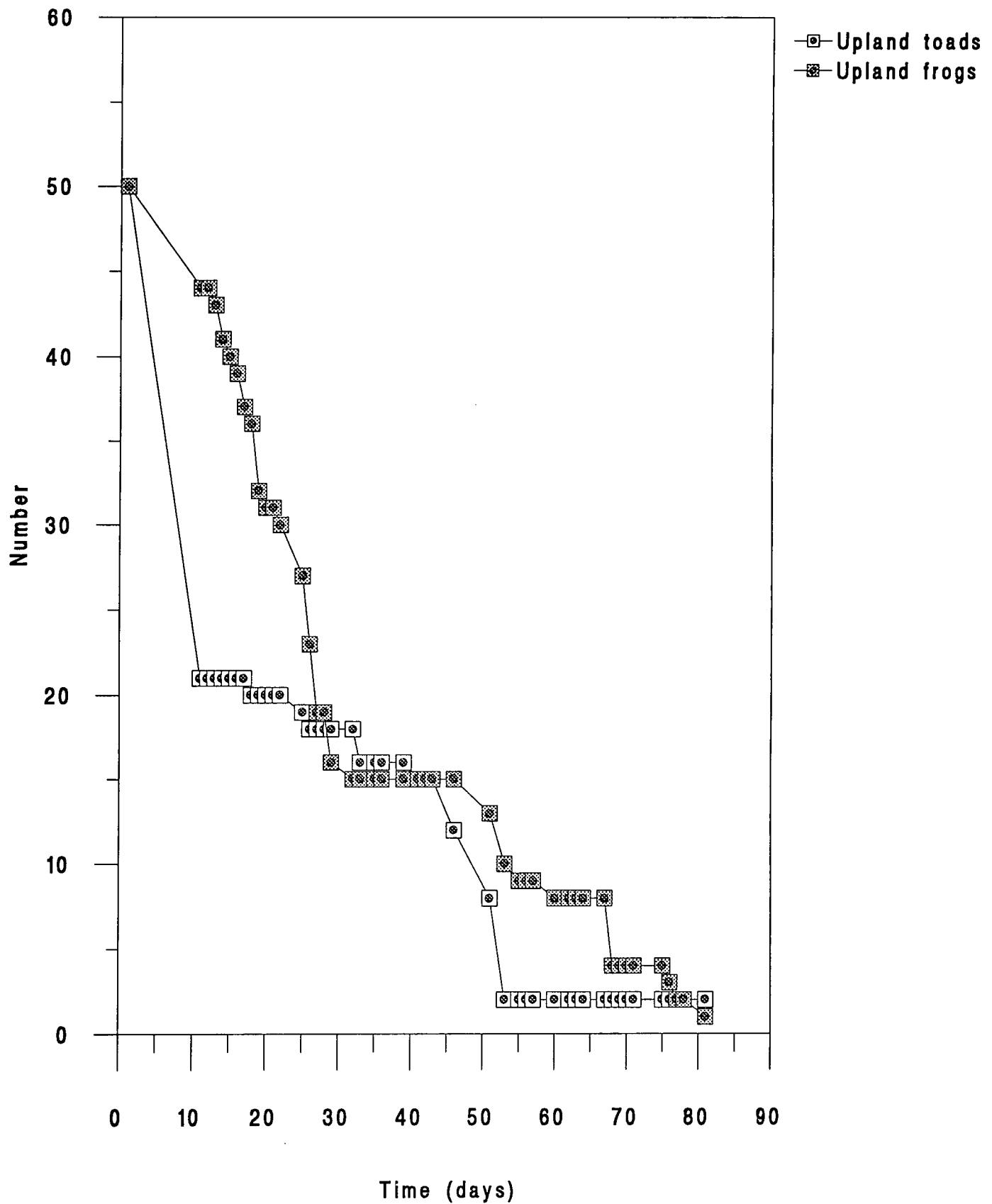


Figure 28. The Survival of Upland Frogs and Toads at 25 °c With Time



At 5°C, 10°C and 25°C toad mortality significantly exceeded that of frogs as can be seen from the Chi-squared values in Table 10 of 42.6, 6.95 and 8.02. However, at 15°C upland frog mortality was significantly greater than that of upland toads with a Chi-squared value of 49.60.

#### 3.2.2.2. A Comparison of the Mortality of Lowland Frogs and Toads at Different Temperatures

Figure 29 shows the mortality of lowland frogs and toads at 5°C. Figure 30 shows the mortality of lowland frogs and toads at 8°C. Unusually no mortality occurred in this population. Figure 31 shows the mortality of lowland frogs and toads at 10°C. Figure 32 shows the mortality of lowland frogs and toads at 15°C. Figure 33 shows the mortality of lowland frogs and toads at 25°C.

Toad mortality was significantly greater at 5°C, 8°C and 25°C with Chi-squared values of 16.17, 129.79 and 11.99 respectively whereas at 10°C and 15°C there was no significant difference between the two populations.

#### 3.2.2.3. A Comparison of the Mortality of Upland and Lowland Frogs at Different Temperatures

Figure 34 shows the mortality of upland and lowland frogs at 5°C. Figure 35 shows the mortality of upland and lowland frogs at 8°C. Figure 36 shows the mortality of upland and lowland frogs at 10°C. Figure 37 shows the mortality of upland and lowland frogs at 15°C. Figure 38 shows the mortality of upland and lowland frogs at 25°C.

At 5°C lowland frog mortality was greater than upland with a Chi squared of 24.86 but at 8°C and 15°C upland frog mortality was greater with Chi-squared values of 61.01 and 11.34. No significant differences emerged between the populations at 10°C and 25°C.

#### 3.2.2.4. A Comparison of the Mortality of Upland and Lowland Toads at Different Temperatures

Figure 39 shows the mortality of upland and lowland toads at 5°C. Figure 40 shows the mortality of upland and lowland toads at 8°C. Figure 41 shows the mortality of upland and lowland toads at 10°C. Figure 42 shows the mortality of upland and lowland toads at 15°C. Figure 43 shows the mortality of upland and lowland toads at 25°C.

The only significant difference evident was at 15°C where the mortality of lowland toads was greater than upland with a Chi-squared value of 29.30.

Figure 29. The Survival of Lowland Frogs and Toads at 5 °c With Time

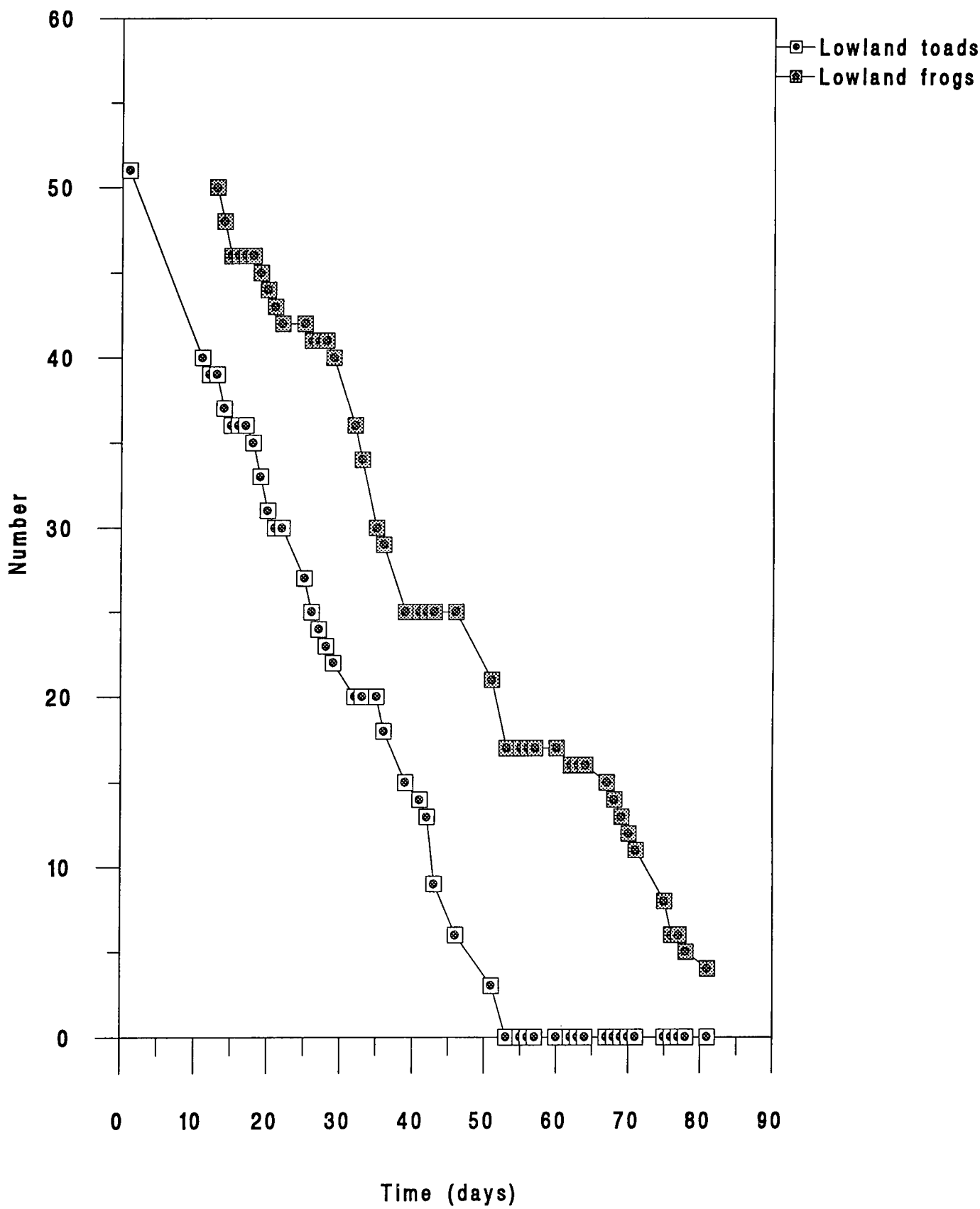




Figure 30. The Survival of Lowland Frogs and Toads at 8 °C With Time

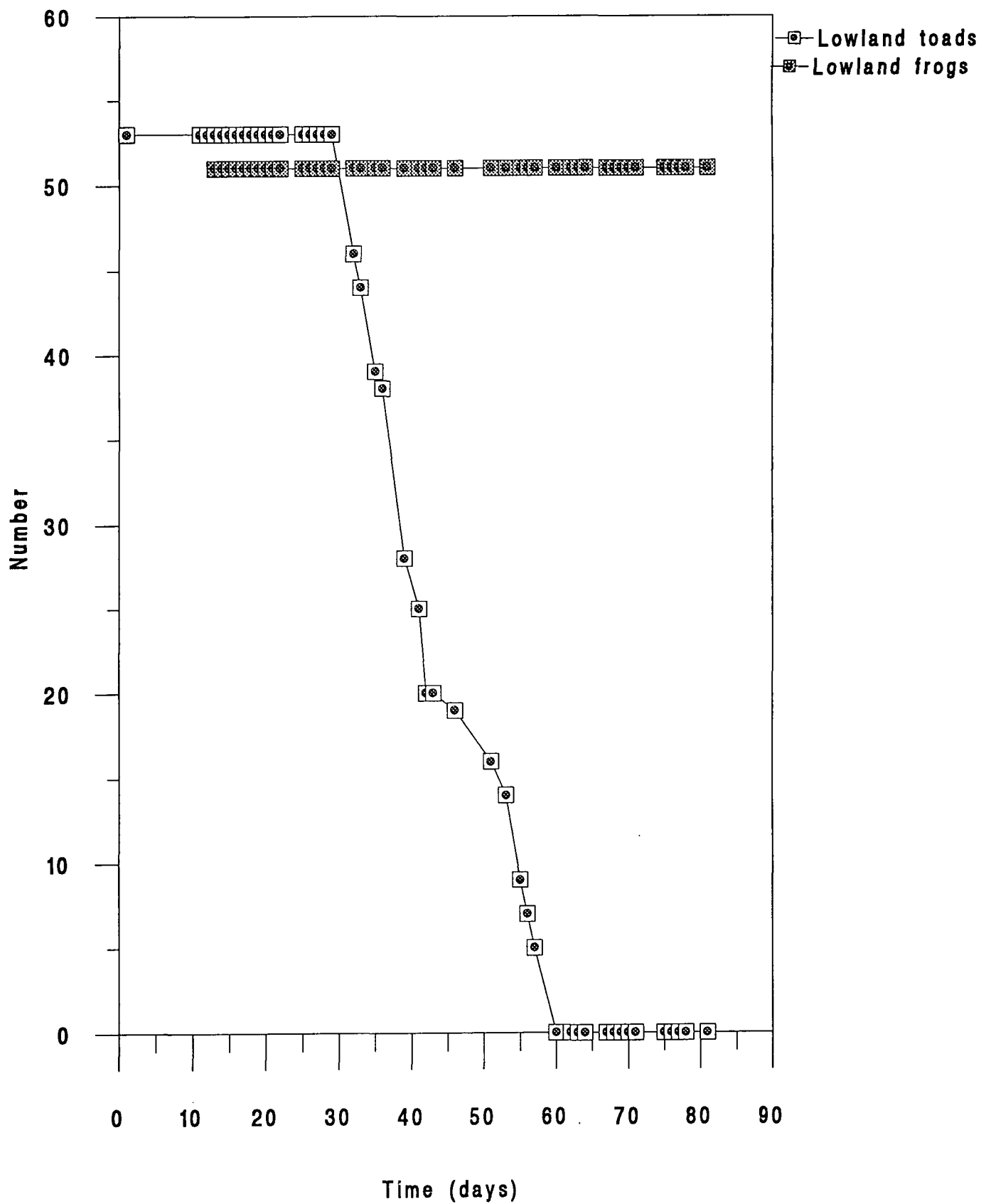


Figure 31. The Survival of Lowland Frogs and Toads at 10 °c With Time

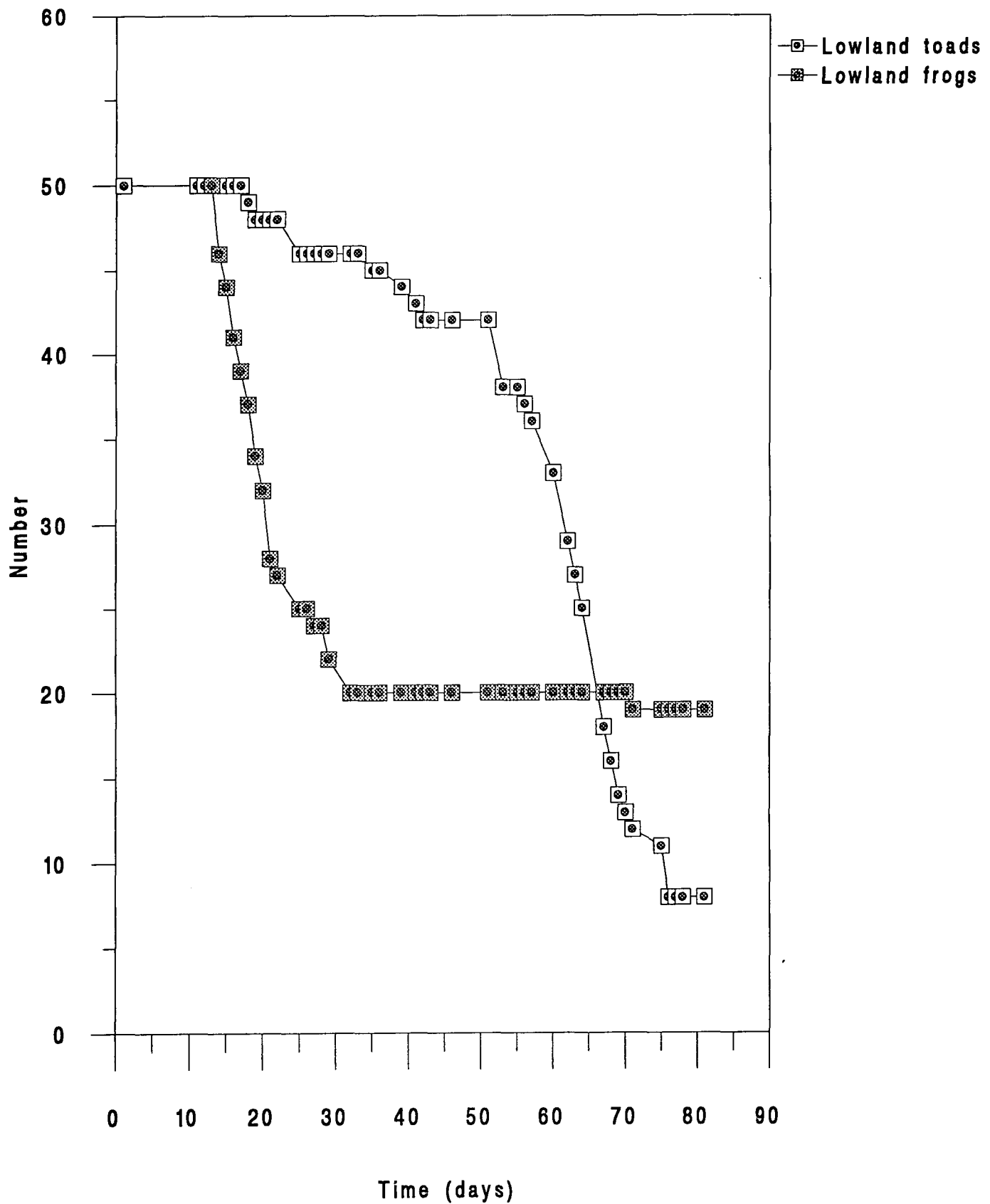


Figure 32. The Survival of Lowland Frogs and Toads at 15 °c With Time

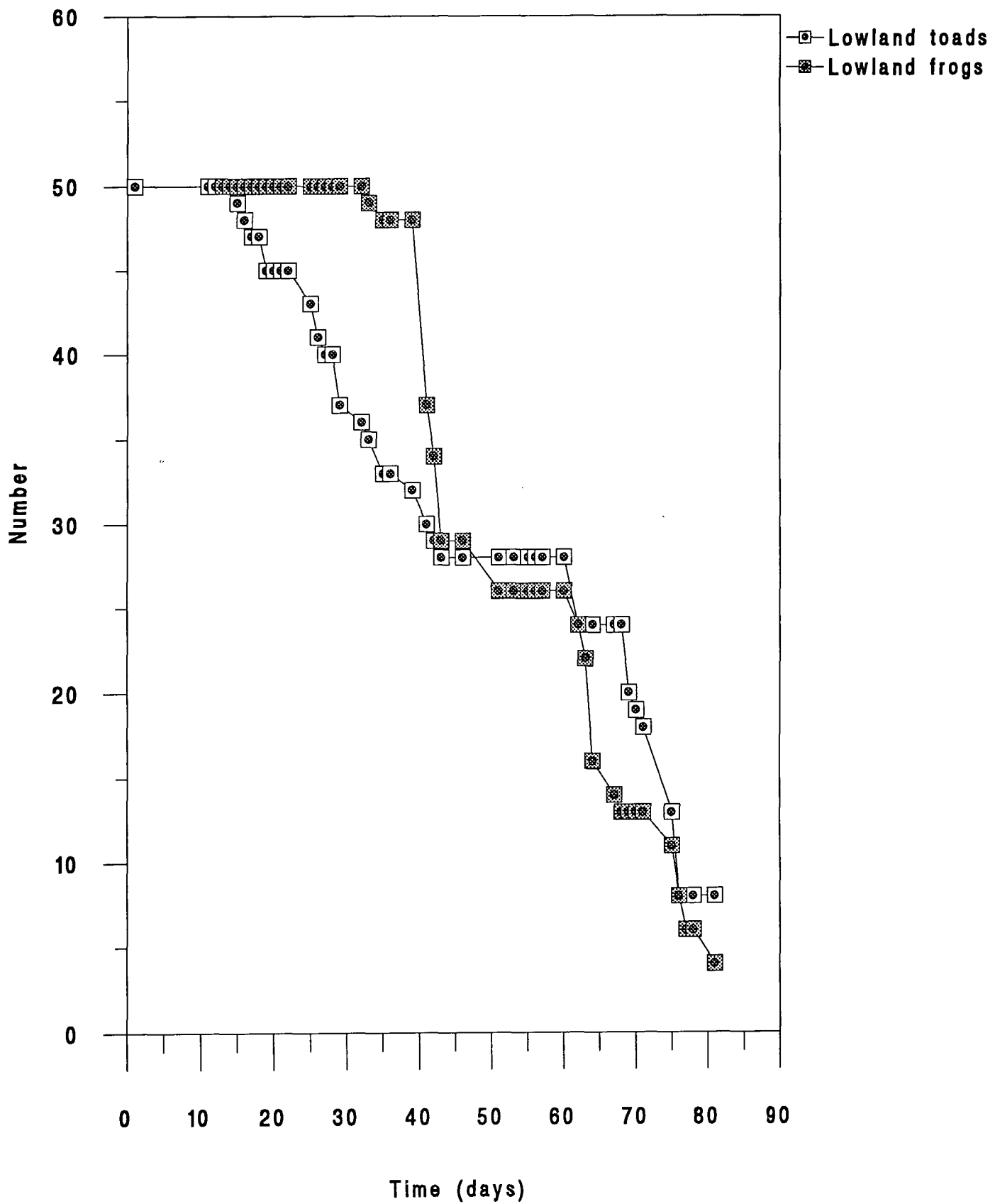


Figure 33. The Survival of Lowland Frogs and Toads at 25 °c With Time

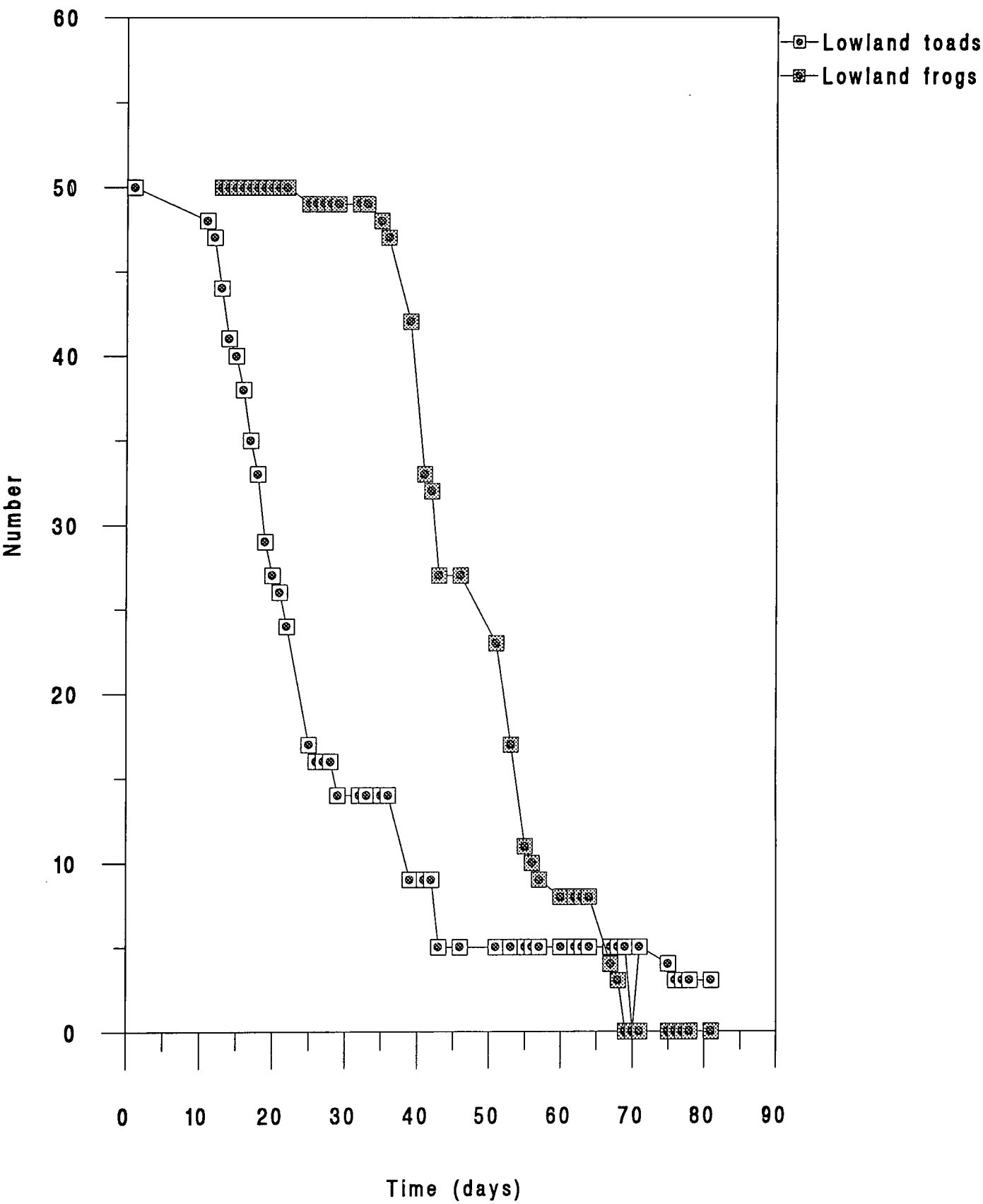


Figure 34. The Survival of Upland and Lowland Frogs at 5 °c

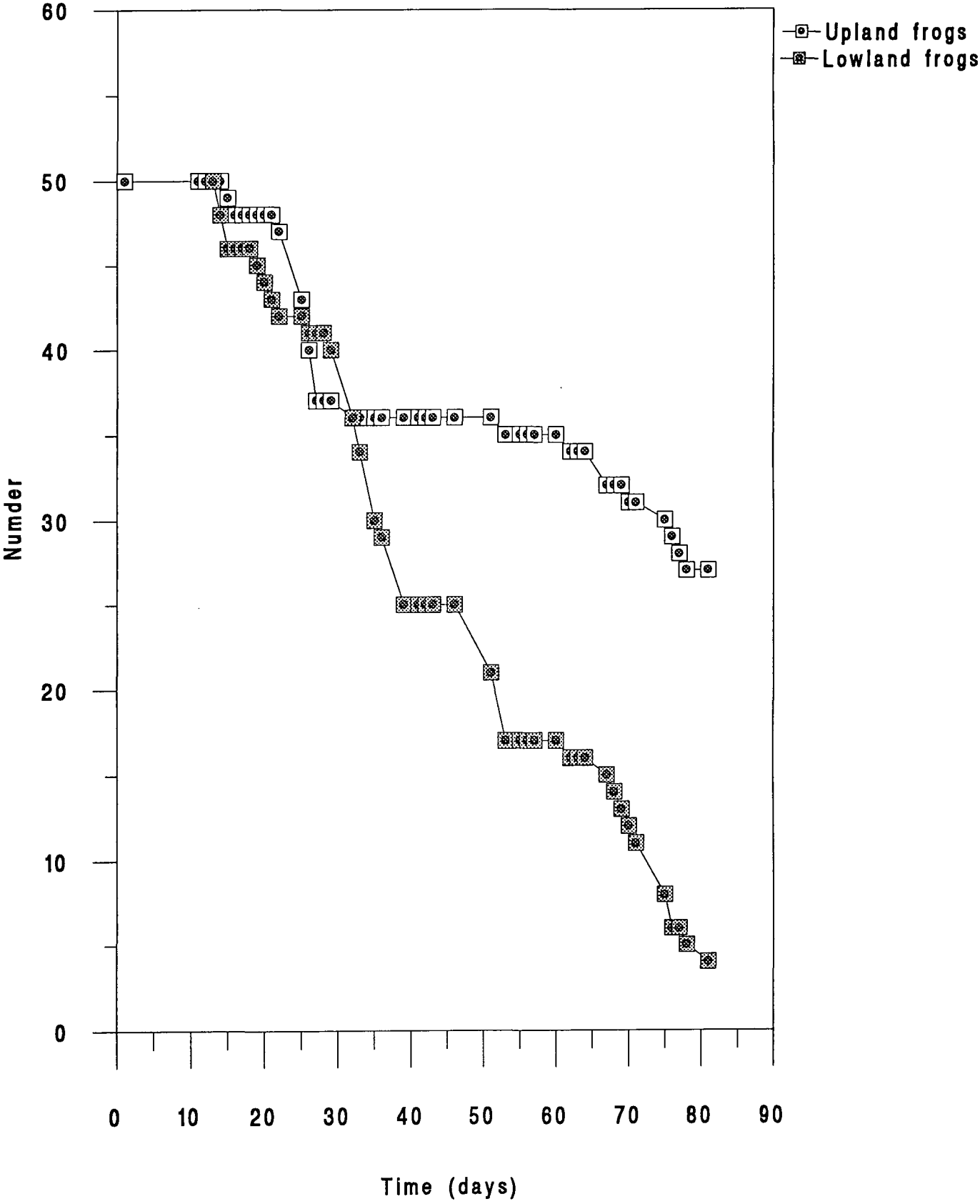


Figure 35. The Survival of Upland and Lowland Frogs at 8°C With Time

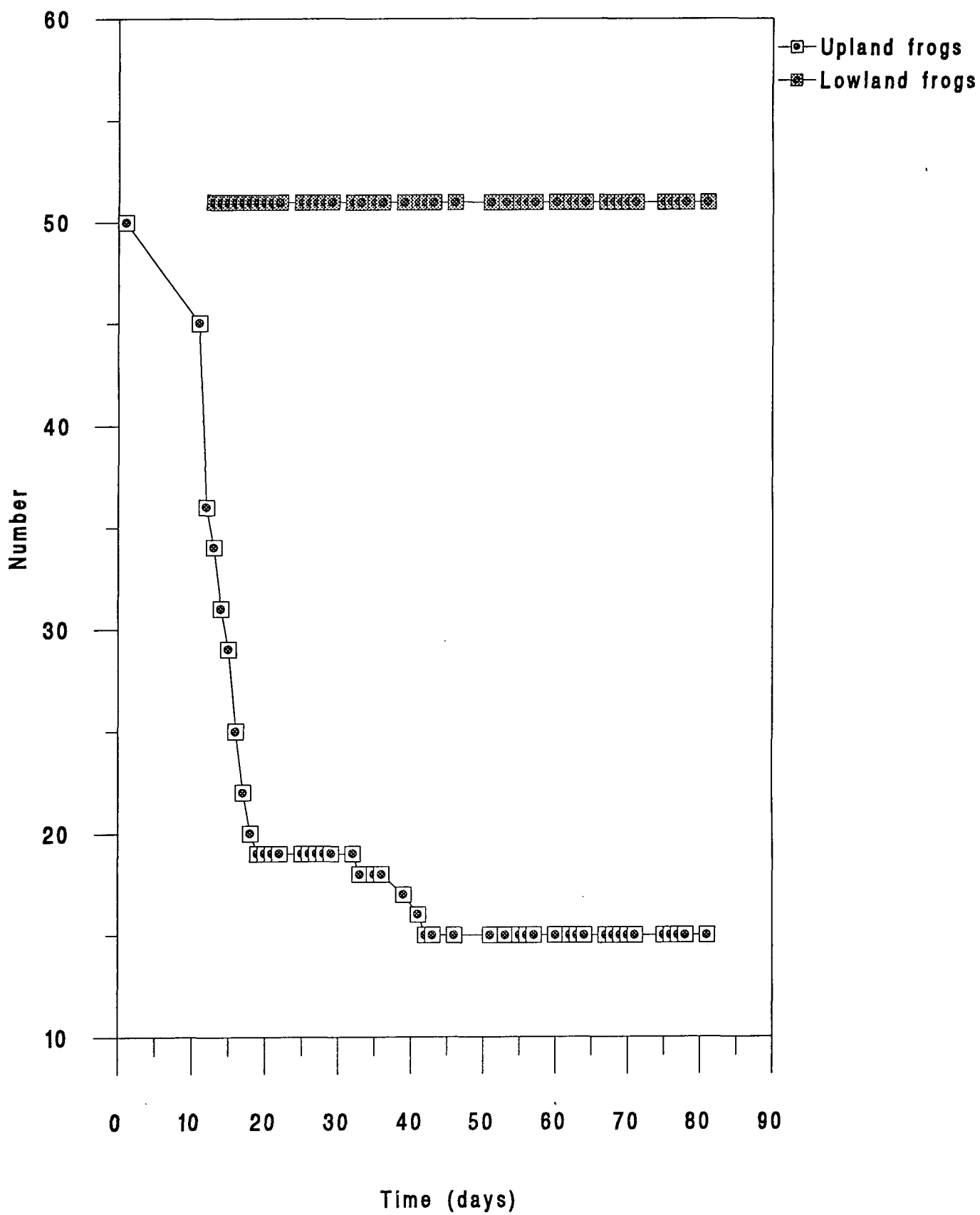


Figure 36. The Survival of Upland and Lowland Frogs at 10 °c With Time

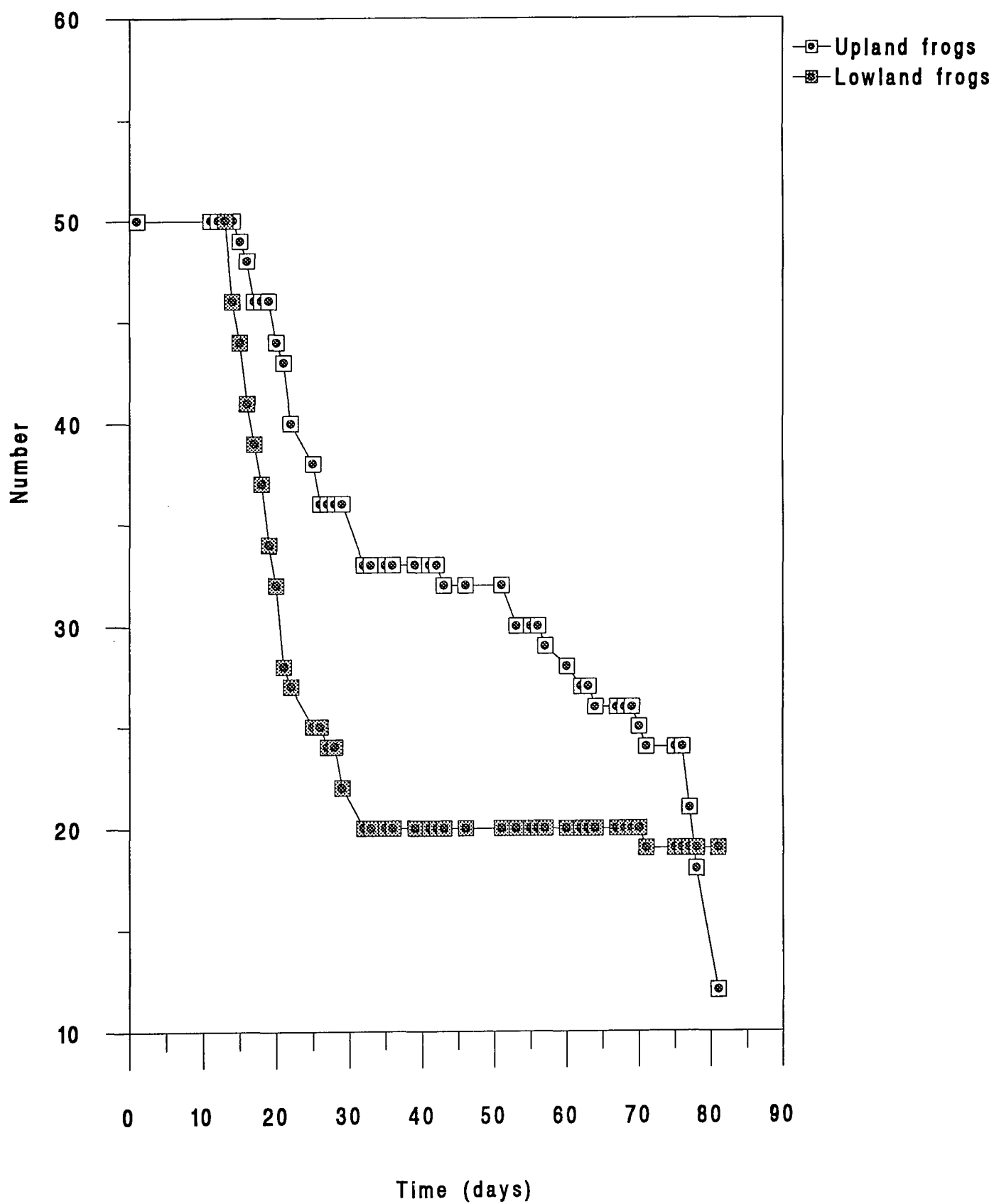


Figure 37. The Survival of Upland and Lowland Frogs at 15 °C With Time

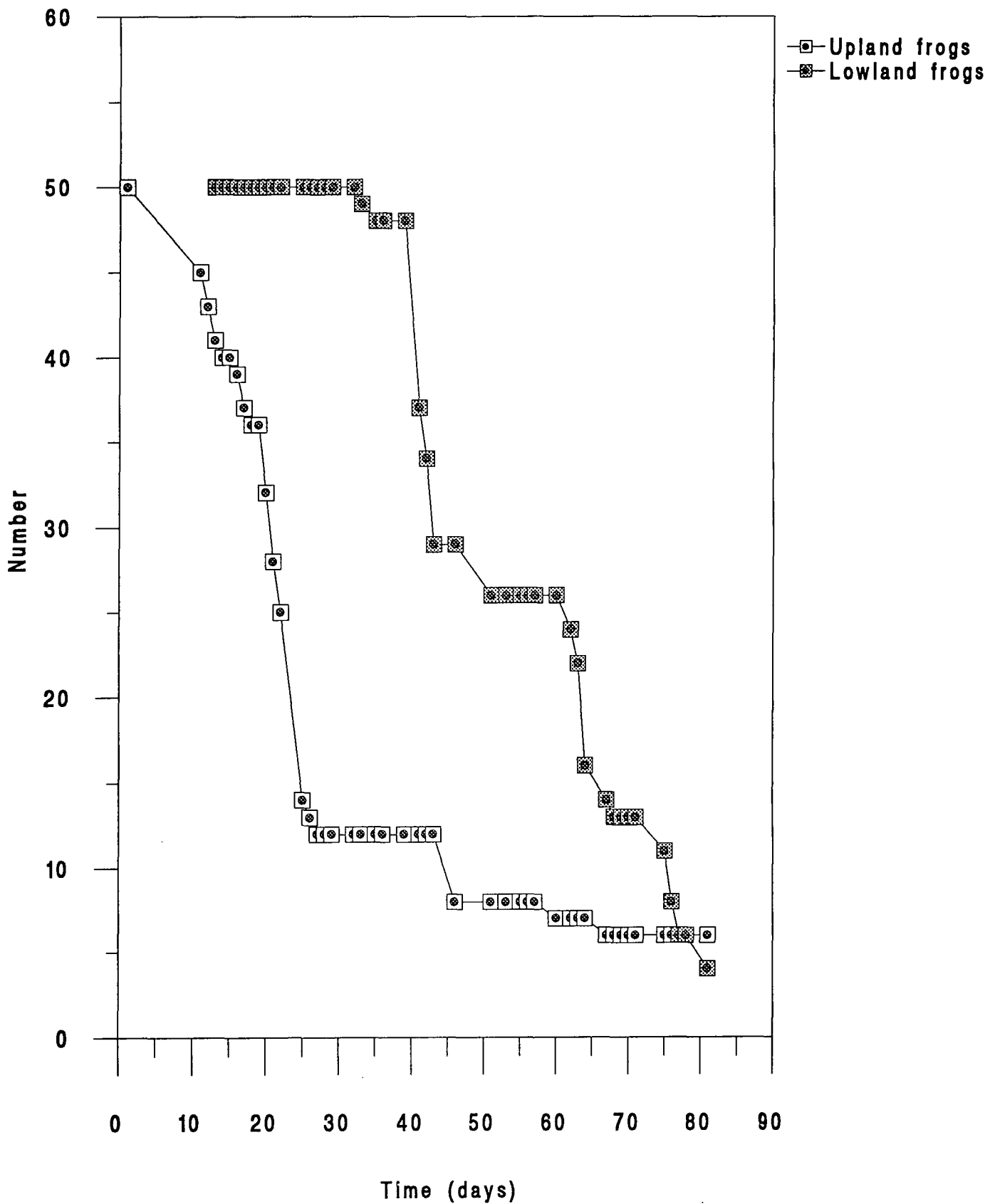




Figure 38. The Survival of Upland and Lowland Frogs at 25 °c With Time

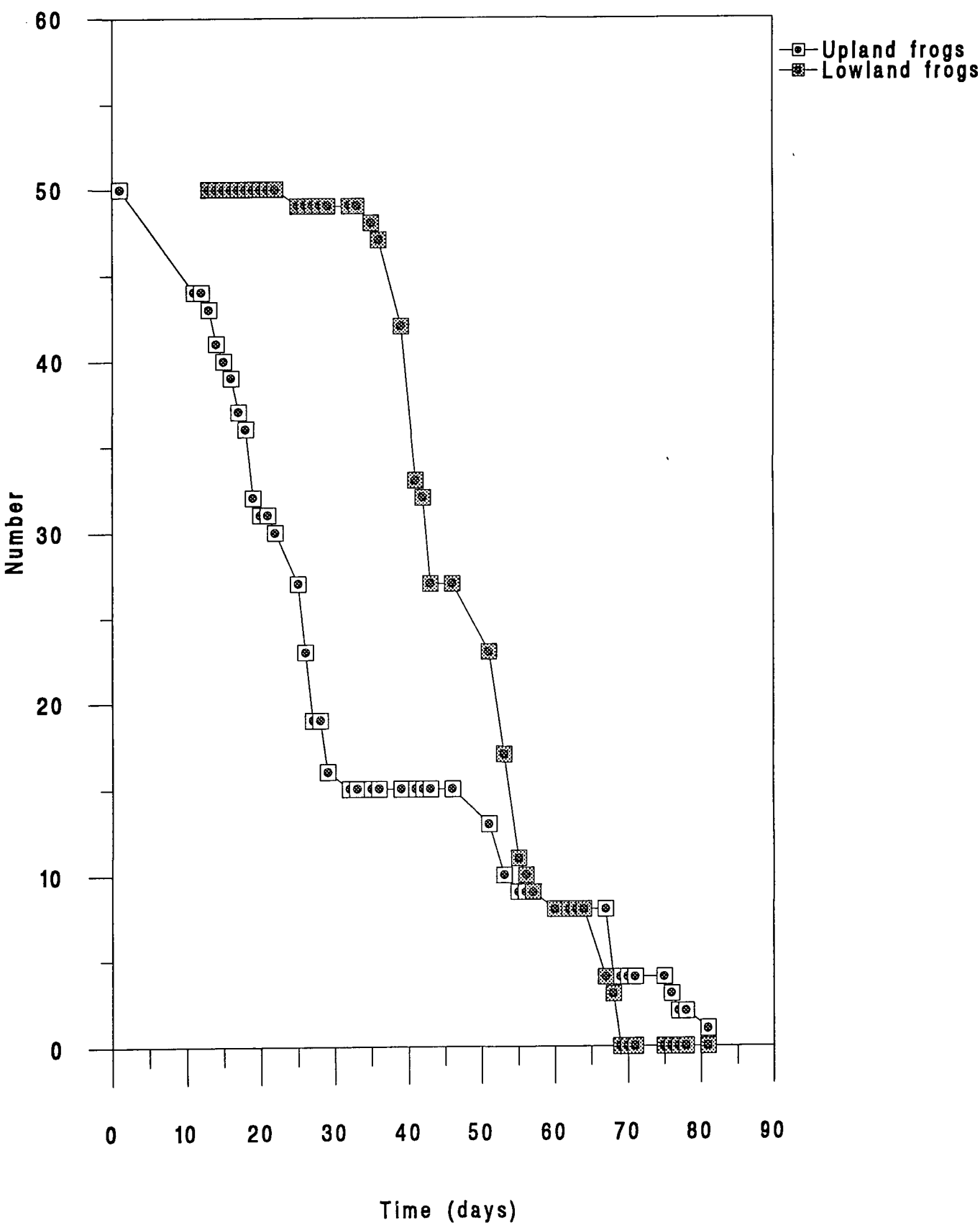


Figure 39. The Survival of Upland and Lowland Toads at 5 °C

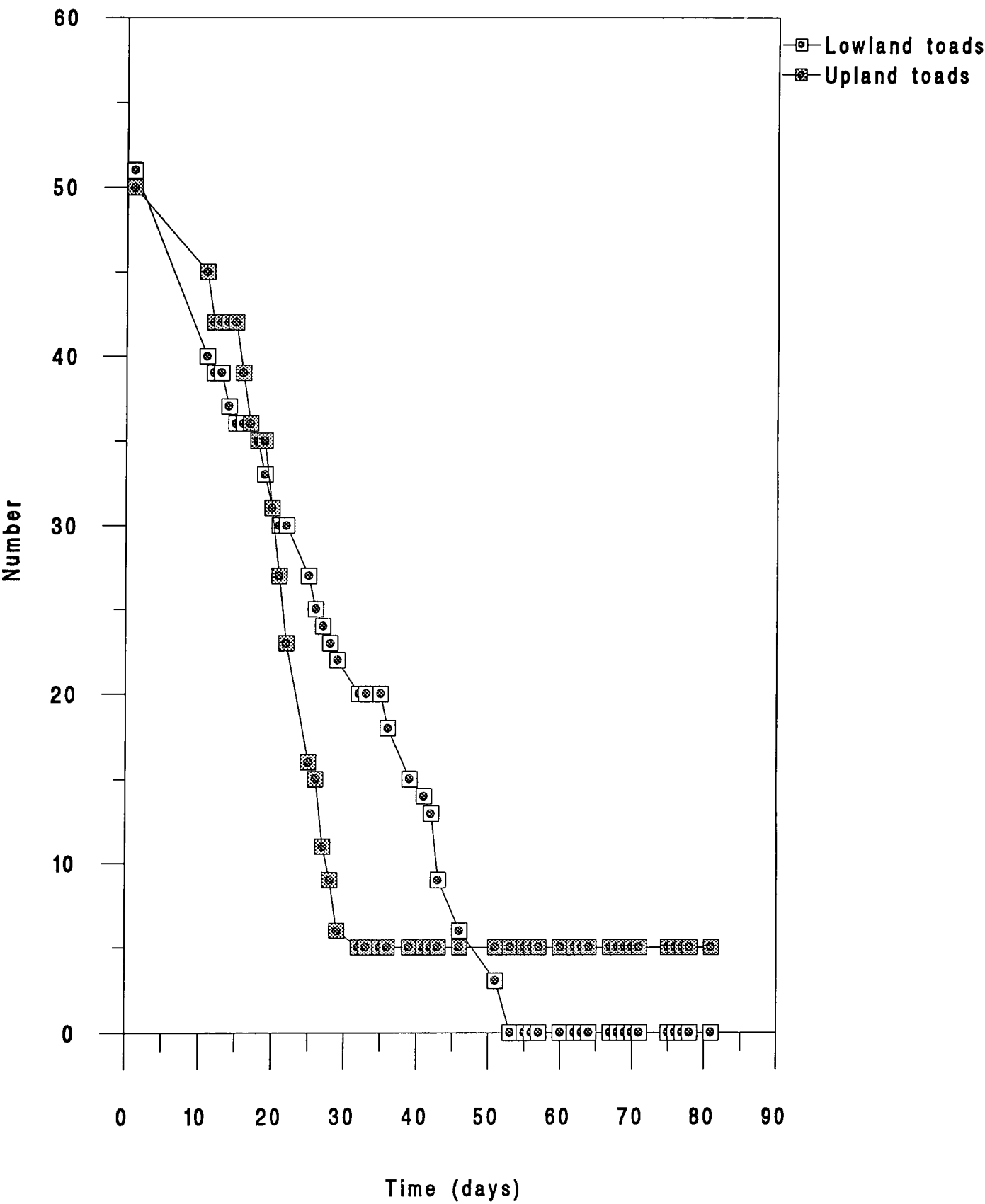


Figure 40. The Survival of Upland and Lowland Toads at 8 °C With Time

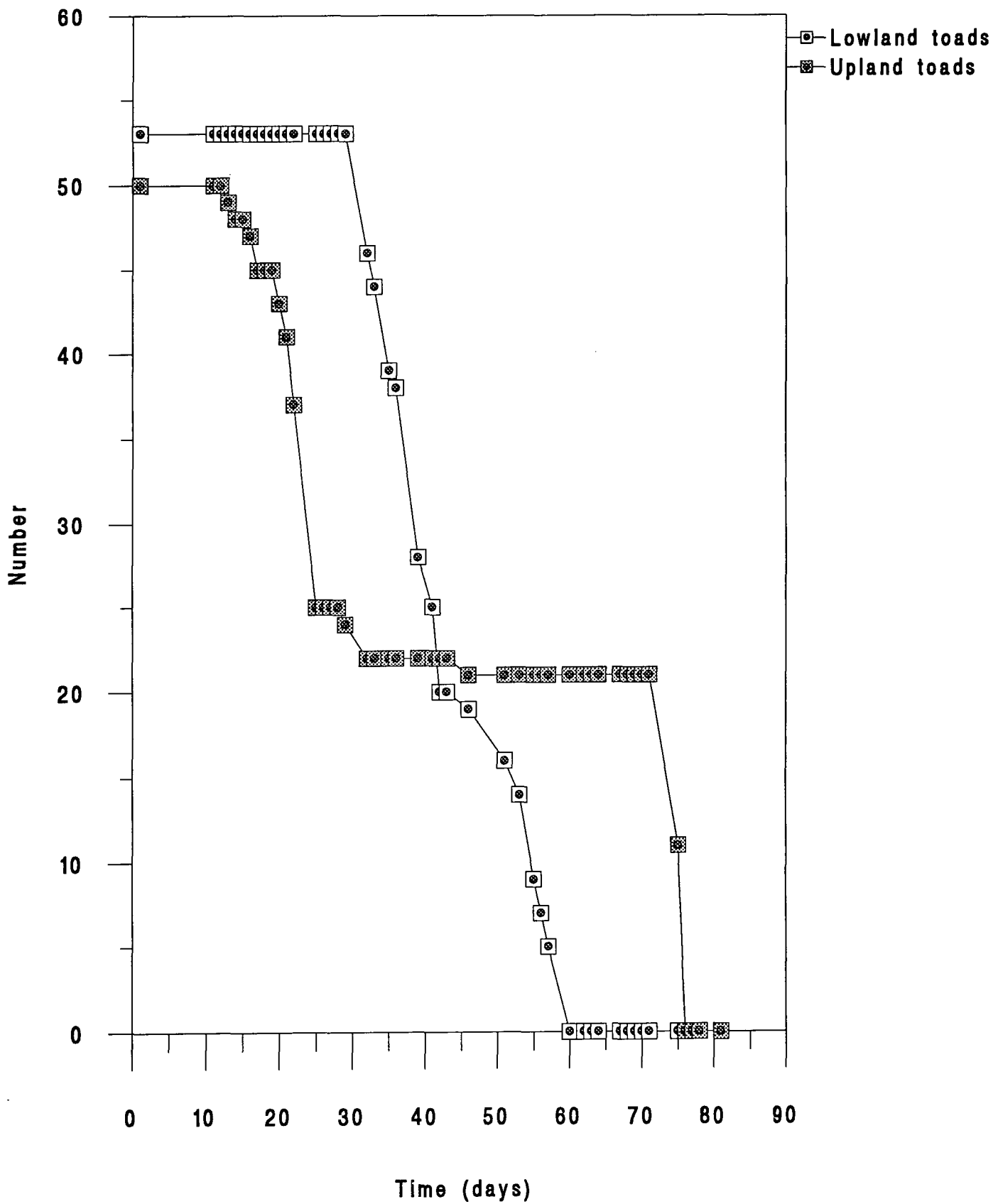


Figure 41. The Survival of Upland and Lowland Toads at 10 °c With Time

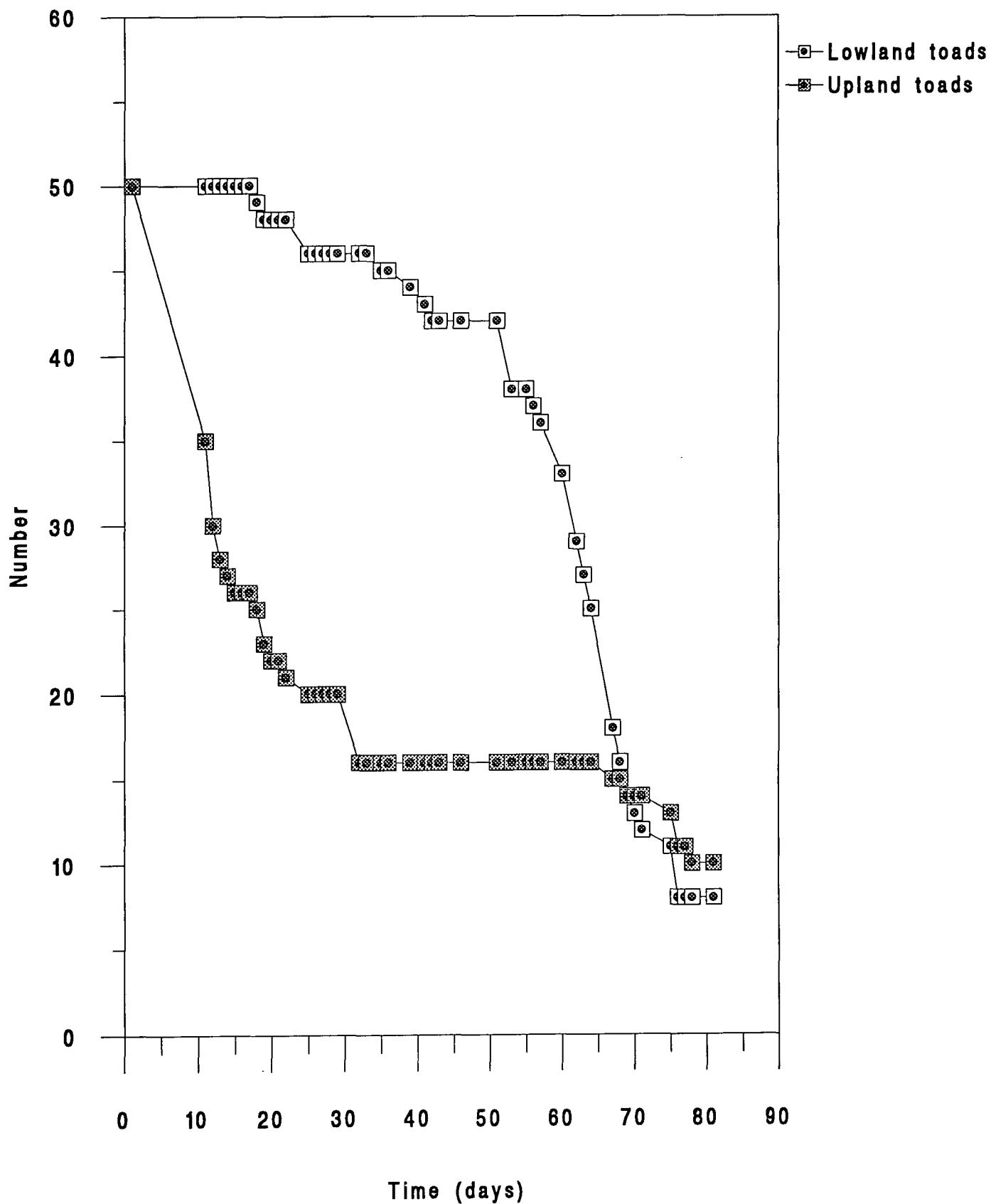


Figure 42. The Survival of Upland and Lowland Toads at 15°C With Time

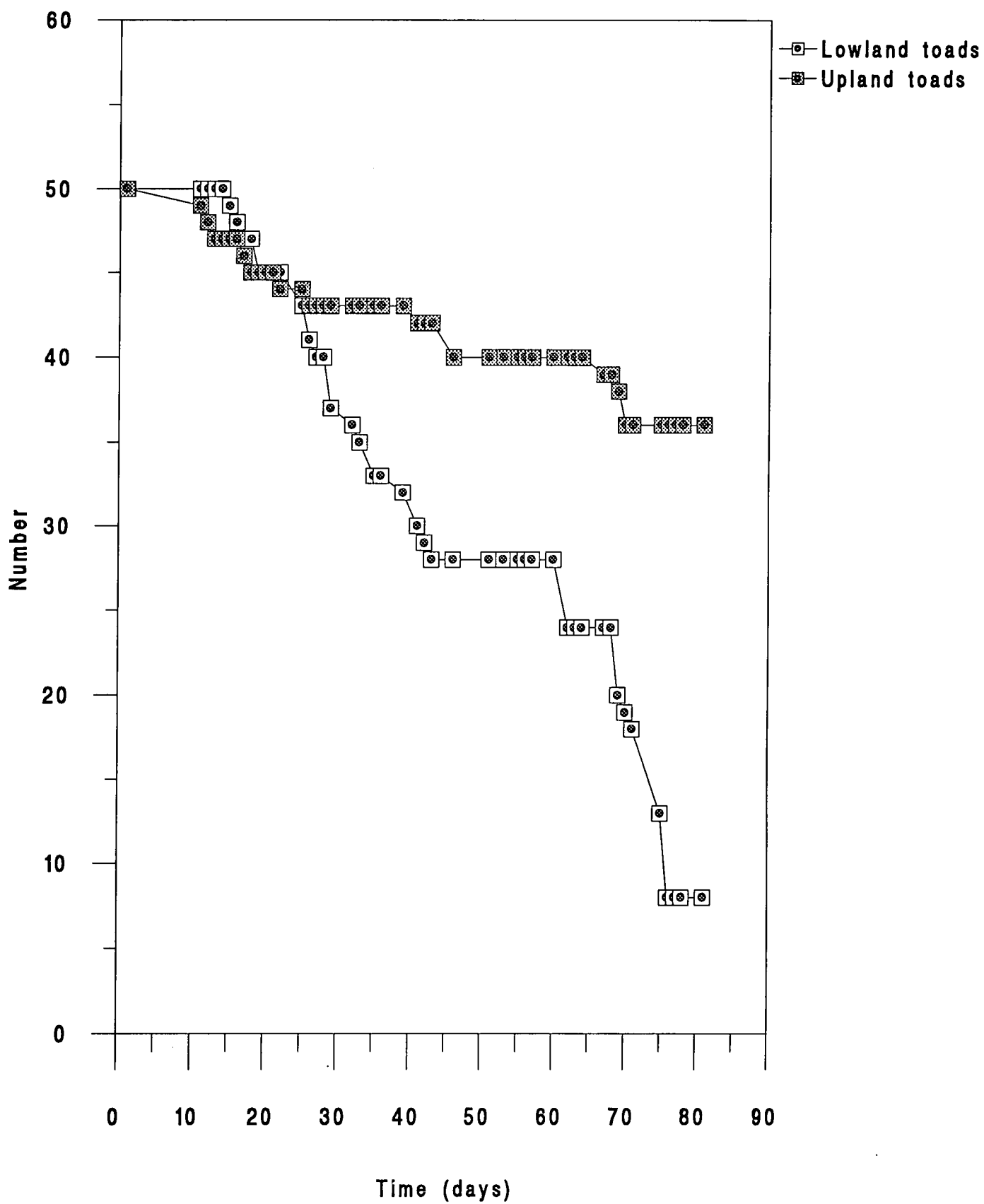
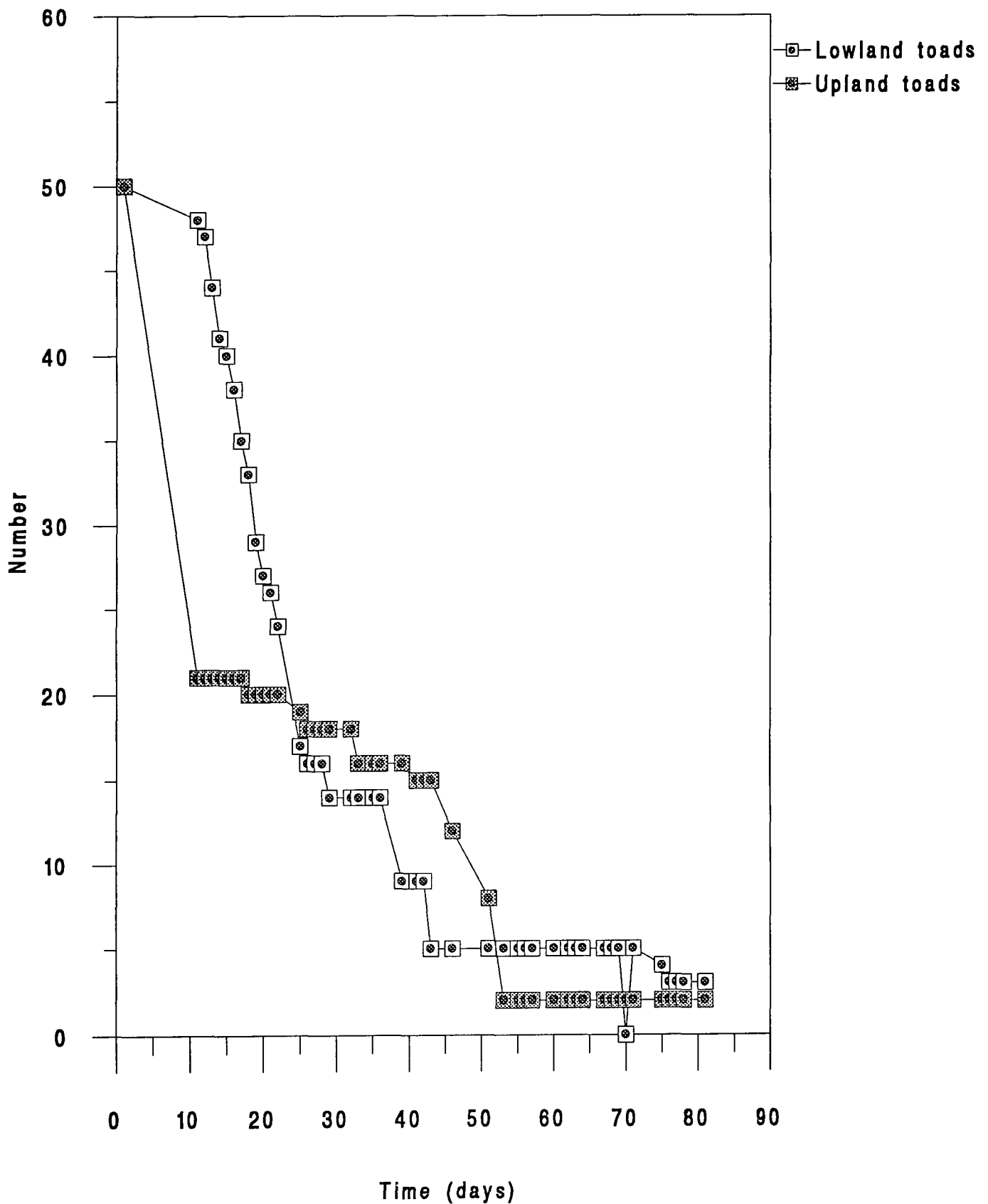


Figure 43. The Survival of Upland and Lowland Toads  
at 25°C With Time



### 3.2.2.5. Summary of the Mortality of Upland and Lowland Frogs and Toads at Different Temperatures

Both upland and lowland toads suffered greater mortality than upland and lowland frogs at the temperature extremes of 5°C and 25°C.

It is difficult to discern any patterns in the mortality exhibited by upland and lowland frogs and toads at the different culture temperatures used.

### 3.3. The Mortality at Different Developmental Stages

The summary table below shows the mortality at particular stages for the four populations at 25°C. Although this is the temperature at which the greatest mortality occurred it was also the only temperature at which the four populations overlapped in enough developmental stages to allow comparisons between the mortality at different stages to be observed.

Table 11. The number of upland frogs, upland toads, lowland frogs and lowland toads dying at particular stages when cultured at 25°C.

Population	Development stage																
	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Upland frogs	7	7	4	4	0	5	1	0	0	0	1	1	1	0	1	-	-
Upland toads	-	-	-	-	-	16	16	0	0	0	9	1	0	4	1	3	0
Lowland frogs	1	0	0	0	0	0	0	1	1	1	0	0	0	0	2	0	0
Lowland toads	24	2	8	5	5	4	0	0	0	1	1	0	0	3	-	-	-

It is difficult to discern any clear patterns from the above table but there does appear to be a general trend of higher mortality in the less advanced early stages and lower mortality when the tadpoles are more developed, particularly by stage 46 i.e. the completion of metamorphosis.

### 3.4. Pond Temperatures

The temperature of pond A was 20.5°C and pond C 18.5°C. Pond C had dried up when the temperatures were taken and therefore no record is available. These recordings confirm that lower pond temperatures are experienced at higher altitudes.



## Chapter 4.

### Discussion

#### 4.1. Upland and Lowland Frog and Toad Development Rates at Different Temperatures.

The geographical distribution of all organisms is principally governed by climate particularly temperature and precipitation. Cooler temperatures are experienced at higher latitudes because radiant energy from the sun has to cover a larger area as it hits the earth at a more obtuse angle and also has to pass through a greater distance of atmosphere at higher latitudes.

The order Anura are the most successful order of Lissamphibia and display the greatest taxonomic and ecologic diversity. They are present on all continents except Antarctica and occur on most continental islands such as Japan and the Seychelles. The family Ranidae (true frogs) has its greatest diversity in the tropics and appears to have originated here but its distribution is almost cosmopolitan due to the genus *Rana* which is widely distributed in temperate parts of Europe, Asia, North America, Middle America and the northern half of south America. The family Bufonidae (true toads) is also almost cosmopolitan due to the success of the genus *Bufo* which has spread throughout all habitable continents except Australia (Porter, 1972).

Amphibians are particularly susceptible to desiccation and require a constant source of freshwater. They have little tolerance for salt water which means that oceans are a substantial barrier to their dispersal. Amphibians are ectothermic i.e. they rely on external heat sources to maintain their body temperature and therefore their activity is strongly governed by environmental temperature. Many ectotherms therefore display behavioural responses in order to regulate their body temperature. For example, many species of grasshoppers and locusts climb vegetation to bask in the early morning sun and orientate their bodies so as to maximise heat absorption. When they have achieved their preferred temperature they move to face the sun to avoid overheating. Reptiles also exhibit behavioural thermoregulatory responses which at its simplest involves shuttling between sun and shade. Some reptiles, such as the Galapagos marine iguana also utilise physiological mechanisms to maintain body temperature. This animal must dive into the sea in order to feed on marine algae; to prevent excessive cooling by the water the iguana displays peripheral vasoconstriction and a reduction in heart rate on entering the water to reduce blood flow and thus

heat loss. On return to the land increased heart rate and peripheral vasodilation occur to rapidly rewarm the animal to its preferred temperature (Cossins and Bowler, 1987).

Except for water, the most absolute barrier to amphibian dispersal at the present time is cold but because they have the ability to hibernate underwater or in burrows, and thus avoid winter climatic extremes, summer temperatures are the most important in governing their dispersal if suitable hibernation sites are available.

Temperature thus has an important effect on the distribution of frogs and toads. The geographical distribution of the genus *Rana* is closely related to its temperature tolerance. For example, if we look at four species of North American frogs; *Rana clamitans*, *R. palustris*, *R. pipiens* and *R. sylvatica* we find that *R. sylvatica* which has the northernmost geographical limit is also the most cold-tolerant in the adult stage. An additional correlation with distribution is that frogs that breed at low environmental temperatures have low minimum and maximum temperatures for breeding and for embryonic development too (Moore, 1939). Physiological differences in response to temperature also occur within species such as *R. pipiens* which cover an unusually wide geographic range; embryos of northern species develop faster at lower temperatures than southern species (Moore, 1949a).

In this investigation upland frogs developed faster than upland toads at lower temperatures but the upland toads displayed a more marked increase in development with increasing temperature and thus developed faster than upland frogs at higher temperatures.

When collected from the wild the upland toad population was at stage 14 and upland frogs were at stage 18. One can therefore make a rough calculation of the difference in spawning date of the upland frog and toad adults. Beattie (1987) measured the mean temperature of the water in the spawning sites of the Common frog in 1976. He found that in this year the mean water temperature between the onset of spawning and hatching was 7.1°C in a lowland pond (altitude 61m), 1.1°C higher than the mean temperature in an upland pond (altitude 556m). If we assume a linear relationship between temperature and altitude, the mean temperature in pond C would be approximately 6.4°C. According to my data in Appendix A. upland toads will take 22 days to develop from stage 14 to 18 at 5°C and 15 days at 8°C and therefore at 6.4°C one could estimate that the time taken for upland toads to develop from stage 14 to 18 would be approximately 18 days and

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this therefore gives a rough estimate of the date of toad spawning to be 18 days later than frogs at this altitude. Thus the upland frog embryos and tadpoles develop earlier in the season than the toads when the temperature is colder and these populations have therefore adapted to grow faster at the lower temperatures that they are normally exposed to. In contrast, the toads experience warmer temperatures during their development period and are thus better adapted and grow faster at higher temperatures. An additional factor explaining the more rapid development of upland toads at higher temperatures may be that because they spawn almost three weeks later than frogs substantially less time is available to complete metamorphosis before winter and they have evolved a more rapid rate of development through natural selection to compensate for this. Differences in the duration of the larval period have been observed in other amphibians and reflect adaptation to the embryonic environment. Typically, amphibians breeding in temporary waters have a short larval period because natural selection has favoured rapid development and early metamorphosis before complete evaporation of the breeding water occurs (Porter, 1972).

It was noticeable when collecting tadpoles for this investigation that the Common toad had a much more restricted altitudinal distribution than the Common frog; despite extensive searching toad tadpoles were not found in any ponds above 225m whereas frogspawn has been reported at altitudes in excess of 600m in the particular area being used to collect tadpoles for this investigation (Beattie, 1977).

The distribution of amphibians in Northumberland was investigated by Durkin and Cooke (1984) and their occurrence was noted to be generally similar to their national status. They found that the Great Crested Newt, *Triturus cristatus*, was restricted mainly to two localised areas in the eastern part of the county on mixed agricultural land. *Rana temporaria* was the most common of the amphibians recorded being present in 67.1% of the ponds surveyed. The Common toad, *Bufo bufo*, was located in 30.9% of the surveyed ponds and was found mainly in lower altitude ponds in the river valleys and on the coastal plain.

One could speculate that a possible explanation for the Common toad being restricted to lower altitudes is that it has a more limited genetic capacity to adapt its development rate than the frog. The summary graph for the development of upland frogs and toads at different temperatures indicated that the development of upland toads increased more rapidly with increasing temperature

than did the development of upland frogs which suggests the upland frogs are compensating for the increased temperature and thus may have a greater genetic capacity to adapt to different temperature regimes. In addition the tadpoles of the Common toad have less time for development because the adults spawn later than frogs and thus above a particular altitude spawning would be too late to leave enough time for the tadpoles to grow and metamorphose. This characteristic would presumably continue up the altitudinal gradient and may indicate that toadspawn/tadpoles are less able to withstand low temperatures than those of frogs and thus toads delay spawning until more favourable weather conditions arrive. As altitude increases temperature decreases (Manley, 1936). Beattie (1985) measured the water temperature in a series of ponds in the Wear Valley over a four year period and found that the temperature decreased by 1°C for every 270m increase in altitude. If toad embryos are less hardy than those of frogs it may be this factor that ultimately limits their altitudinal distribution.

In contrast to upland frogs and toads, lowland frogs developed faster than lowland toads at higher culture temperatures. This finding is in agreement with Douglas (1948) who observed the development rate of lowland *Rana temporaria* and *Bufo bufo* embryos from the time of first cleavage to stage 20. He found that at the three temperatures of 13.9°C, 17.5°C and 24.0°C used in the experiment the embryos of the Common frog developed more rapidly than those of the Common toad.

It was hypothesised earlier in this discussion that upland toads developed more rapidly than upland frogs at 15°C because this is the temperature to which they are best adapted. It was also suggested that the toads were developing faster to compensate for their more limited growing season. Although the same is true at lower altitudes i.e. frogs spawn earlier in the season than toads, both populations spawn earlier than their upland counterparts and therefore the same selection pressure for the more rapid development of toads may not be present as at these lower altitudes the growing season is longer and development is more rapid because the average temperature is higher. This was confirmed by the temperature readings taken at ponds A and C; pond C. had an altitude of 225m and a water temperature of 18.5°C whereas pond A at 60m altitude had a water temperature 2°C higher than this on the same day and at approximately the same time.

No significant difference was observed between the development rates of upland and lowland frogs. Beattie (1987) found that embryos from lowland frogs (mean altitude 187.0m) developed approximately 1% faster than those from upland frogs (mean altitude 621.2m) at 16°C but approximately 4% slower at 6°C i.e. lowland frog embryos developed more rapidly than upland at higher temperatures but at lower temperatures the upland embryos developed more rapidly. One would therefore expect the lowland embryos in this study to grow more rapidly at 25°C but it was found that there was no significant difference between the development rates of these two populations. However, my upland site (225m) is only 38m higher than Beattie's lowland site and this may explain why there is no difference between the development of upland and lowland frogs. One could therefore speculate that the upland frogs used in my investigation are still at a low enough altitude that an adequate growing season and average temperature is available to allow the completion of metamorphosis and thus there is no strong selection pressure for more rapid development of upland tadpoles at lower temperatures or less rapid growth at higher temperatures.

Upland toads developed more rapidly than lowland toads at the two highest incubation temperatures used in this investigation and displayed a more pronounced increase in development rate with increasing temperature. A similar hypothesis as previously forwarded appears to offer the most likely explanation for such results. That is that the upland toad tadpoles develop more rapidly than those from lowland sites to compensate for the fact that normally they have to grow at a lower temperature which inhibits development and also have a shorter period in which to metamorphose and build up fat reserves before Winter arrives.

It was concluded that there was no difference in the development rates of upland and lowland frogs but there does appear to be a difference between upland and lowland toads as described above. This would appear to be related to the fact that toads spawn later in the Spring than frogs. At the upland site although the frogs spawn later than the lowland counterparts the time available for growth is still sufficient to enable the completion of metamorphosis. However, upland toads spawn even later than upland frogs and therefore they are subjected to greater selection pressure to complete metamorphosis as less time is available and thus they have evolved to develop more rapidly.

An increase in temperature usually leads to increased animal activity due to the increase in kinetic energy of molecules leading to increased rates of metabolism and other processes. Thus

development should be more rapid at higher temperatures and indeed this is what was found during this investigation. There is, however, an upper thermal limit to this rate effect of temperature above which biological processes become progressively more affected by the debilitating effects of temperature. Thus there is an optimal temperature for the most rapid rate of development.

Genotypic or evolutionary adaptations to temperature are displayed in many animals and best demonstrated by studying latitudinally or altitudinally separated populations of the same species.

Vernberg and Costlow (1966) studied fiddler crabs of the genus *Uca* which have a wide geographical distribution. *U. pugilator* occurs along most of the eastern seaboard of North America and they found that populations of this animal from Massachusetts, North Carolina and Florida had significantly different rates of oxygen consumption. Thus crabs from more northerly latitudes displayed higher rates of oxygen consumption than those from lower latitudes when reared under the same conditions.

Dunlap (1980) noted interspecific differences in the metabolism of two species of frog in South Dakota correlated to their lifestyles. When acclimated to 15°C or 25°C, the metabolic rate of *Pseudocaris* was approximately twice that of *Acris*. *Acris* is active through the day and night often basking in the sun whereas *Pseudocaris* is mainly nocturnal, breeds earlier than *Acris* and has a lower tolerance to higher temperatures. These differences therefore imply that *Pseudocaris* is exposed and physiologically adapted to colder conditions than *Acris*.

Although increased temperature leads to an increase in development rates it may also result in greater mortality and therefore this aspect of the upland and lowland frog and toad populations was also investigated.

#### 4.2. The mortality of upland frogs and toads at different temperatures

The pattern of upland frog and toad mortality is irregular although upland toad mortality is significantly greater than that of upland frogs at the temperature extremes of 5°C and 25°C.

As described previously frogs spawn earlier than toads and it was estimated that at pond C the upland frogs spawned approximately 18 days before the upland toads. Thus frog tadpoles are subjected to lower temperatures and may have therefore adapted through natural selection to be more tolerant of low temperatures. These results offer further evidence to support the hypothesis proposed

earlier in this discussion that it is the inability of toad embryos or tadpoles to tolerate low temperatures that limits their upland distribution. Obviously further work involving the lower lethal temperatures for Common frog and Common toad embryos would be required to substantiate this claim.

Lowland frogs and toads exhibited similar mortality characteristics to upland frogs and toads in that toad mortality was significantly greater than that of frogs at the extreme temperatures of 5°C, 8°C and 25°C. The same reasoning can also be applied i.e. because frogs spawn earlier in the year they are more frequently subjected to lower temperatures and have therefore adapted to survive better at such temperatures.

The higher mortality of upland and lowland toads at 25°C compared to upland and lowland frogs at the same temperature may be related to their pigmentation. The black pigmentation of toads will absorb more light and therefore heat than the brown speckled colouration of frog tadpoles and thus may increase their rate of development. However, the conditions encountered in this investigation of high temperature (25°C) and 18 hours of light per day may have combined to cause excessive mortality because the tadpoles were absorbing too much heat leading to the death of large numbers. Obviously such conditions as these would rarely be experienced in the wild.

The decrease in oxygen concentration with increasing temperature as described earlier may also increase mortality at higher temperatures.

The percentage of the four populations surviving at 25°C is very low for all the cultures. This could be related to oxygen demand in two different ways. Firstly the low survivorship could be a direct consequence of less oxygen being available at higher temperatures. The second alternative is that the more rapid development that occurs at 25°C results in a higher oxygen demand and thus increases mortality as insufficient oxygen is available to enable all of the tadpoles to grow rapidly.

Lowland frog mortality was greater than upland at the lowest culture temperature. The same reasoning can be applied as to the greater mortality of upland and lowland toads compared to frogs at 5°C; the upland frog embryos and tadpoles experience lower temperatures during development at higher altitudes and thus natural selection has favoured those which survive better at low temperatures.

Beattie (1987) investigated ecophysical differences between populations of the Common frog from different altitudes in northern England. He found that the lower lethal limit for upland frog embryos (mean altitude 649.2m) was 2.8°C, 1°C lower than the limit for lowland embryos (mean altitude 152.3m) and the upland embryos are therefore better adapted to withstand low temperatures than lowland embryos.

The only significant difference in the mortality of upland and lowland toads occurred at 15°C at which temperature lowland toad mortality was the greater of the two.

Therefore, unlike upland and lowland frogs, no differential mortality was observed between upland and lowland toads at 5°C. This may be explained by the fact that upland toad spawn is laid later in the Spring and therefore does not experience the same low temperatures as upland frogspawn and thus the selection pressure for better survival at lower temperatures is probably not as strong.

The mortality comparisons described in the previous section have assumed all stages to be equally susceptible to mortality. Grainger (1959) observed that gastrulation was the most vulnerable stage in early development. It is difficult to make any firm conclusions regarding the susceptibility of particular stages to mortality using the data from this investigation because many additional factors may be involved in particular the size of the population during a particular stage and the development stage that the tadpoles were at when taken from the wild; presumably the tadpoles will acclimatise to the particular temperature regime at which they are cultured and thus, for example, tadpoles that were at stage 31 when collected from the ponds are likely to exhibit greater mortality during this stage than those that have been cultured in the laboratory since stage 18.

Bearing this in mind the results in Table 11. do not appear to show that any specific stages are particularly vulnerable to mortality.

#### 4.3. Factors of the Experimental Design Which May Have Influenced Development and Mortality

As temperature increases the amount of dissolved oxygen in water decreases and thus the growth of tadpoles may be affected by lack of oxygen at higher temperatures. Krogh (1941) calculated the amount of oxygen contained in freshwater at different temperatures and produced figures of 8.02 ml O<sub>2</sub> l<sup>-1</sup> at 10 °c and 5.57ml O<sub>2</sub> l<sup>-1</sup> at 30°C. Douglas (1948) when studying the



embryonic development rates of frogs and toads at temperatures between 13.9°C and 24.0°C dismissed any differences in oxygenation at different temperatures as having any significant effect on embryonic development because Detwiler and Copenhaver (1940), when studying *Amblystoma* had noted '....eggs can develop, and at a normal rate, with exceedingly low amounts of oxygen in the water.' Clearly the oxygen demand of tadpoles is going to be much greater than embryos and this could constrain tadpole development at higher temperatures. Ideally, therefore, all the aquariums used in this investigation would contain aerators to reduce the possible effect of temperature on the quantity of dissolved oxygen.

An important consideration is the occurrence of adaptive changes in the rate of metabolic processes as a result of temperature change. Therefore animals exposed to increased temperature for extended periods tend to reduce their rate of metabolism to compensate for the rate-increasing effects of temperature changes e.g. *Daphnia* species. This process is known as metabolic compensation and has been documented in frogs (Precht, 1958) and is thought to be advantageous in that it promotes the relative consistency of various rate processes despite seasonal fluctuations in temperatures and thus preserve optimal metabolic performance. As described earlier, the upland frog population studied in this investigation appeared to display some form of compensation in development with increasing temperature whereas the upland toads did not.

When the ponds were revisited on 21 July frogs and toads were found at pond C and toads at pond A. These were all at approximately stage 46; the majority still possessed visible remnants of the tadpole tail and were therefore almost completely metamorphosed. Most of the cultures in the investigation had not completed metamorphosis even at the higher temperatures suggesting that the culture conditions were not optimal. There are many factors which could be responsible for this discrepancy such as overcrowding, competition and inadequate food supply or quality and it is impossible to isolate any one of these without further investigation. There is also the possibility that the fluctuating temperatures experienced in the wild increase growth faster than a constant temperature with the same mean value; again this phenomenon could be studied with additional experiments.

Rose (1960) discovered a feedback mechanism of growth control in *Rana pipiens* tadpoles. He found that the growth rate and size at metamorphosis of tadpoles of this species increased with

the size of the container in which the cultures were reared. In addition, the average growth rate of individuals from the same egg mass cultured in containers of the same size was inversely proportional to the number of individuals. Both these effects were caused by the production of an inhibitory substance produced by the more rapidly growing individuals in the population. Table 1. shows that after 27 days both upland frog and toad populations were approximately equal in size (19 frogs and 16 toads) and therefore any inhibitory effect induced by the more mature individuals would arguably effect both populations equally. If one looks at the population sizes of lowland frogs and toads in Table 3. it can be seen that despite the fact that the lowland frog population was over 3 times that of the lowland toads the lowland frogs still developed much more rapidly indicating that if competition, overcrowding or the production of inhibitory compounds by more mature individuals does have a detrimental effect on tadpole development the effect was negligible in the cultures used in this investigation.

Starvation is also known to have an effect on metamorphosis. Inanition can retard or completely stop metamorphosis until the early stages of hind limb development (D'Angelo et al, 1941).

#### 4.4. Conservation of amphibians

All six amphibians native to Britain have declined in numbers during the past 20 years due to the drainage of fens and marshland together with the loss and acidification of many of their breeding ponds. Cooke (1972) collected data concerning the occurrence of the Common frog and Common toad in the British Isles and found that the frog population declined severely over most of England throughout the 1960's. The root cause of this decline was thought to be loss of suitable wetland habitat due to sites being filled in, drained or physically modified in some way. It was also suggested that the increased use of pesticides had contributed to this decline although a direct link was difficult to prove. Tadpoles readily accumulate fat soluble pesticide residues from the surrounding water (Cooke, 1972). Toad tadpoles are relatively resistant to these residues but they can cause restricted feeding and movement and therefore retard development in frog tadpoles.

A conservation priority is therefore to compensate for this loss of aquatic habitat by creating new ponds. Garden ponds have increased greatly in numbers in recent years and these can provide an

important breeding ground for amphibians, particularly when not stocked with fish which will eat the embryos and tadpoles.

Most amphibians also need areas of moist or shady vegetation such as grassland, woodland or hedgerows to forage for the invertebrates that make up their diet. In addition, hibernation sites such as banks, log piles and the roots of trees are required and therefore such habitats should be conserved.

The destruction of suitable amphibian habitats is not as widespread at higher altitudes due to the unsuitability of such sites for development. Upland sites can therefore provide an important reservoir for amphibian populations, particularly the Common frog as it has an extensive upland distribution. The Common toad, however, is restricted to more lowland areas and thus is more susceptible to the effects of loss of lowland breeding sites. An understanding of the ecology of the Common frog and toad and the possible factors that influence and limit their distribution is therefore important in order to assess the effect of habitat change on these amphibians and thus help to conserve the remaining populations.

### Summary

1. Frog and toad tadpoles were collected from two lowland (altitude <100m) and one upland (altitude >100m) pond and cultured at temperatures of 5°C, 8°C, 10°C, 15°C and 25°C and their mean development stage recorded at regular intervals.
2. Upland frogs developed faster than upland toads from 5°C to 10°C but upland toads developed more rapidly from 15°C to 25°C. It is suggested that upland toads have evolved through natural selection to develop faster at these higher temperatures to compensate for the shorter growing season available to them in the wild.
3. Lowland frogs developed faster than lowland toads at 15°C but at all the other culture temperatures there were no significant differences between the two populations.
4. Upland frogs and toads exhibited equal development rates at 25°C which was the only temperature at which the development rates of these two cultures overlapped.
5. Upland toads grew more quickly than lowland toads at 15°C and 25°C and it is hypothesised that upland toads are adapted to grow faster than their lowland counterparts to compensate for the fact they are laid later in the Spring and have to develop at a lower temperature.
6. The mortality of both upland and lowland toads is greater than upland and lowland frogs at the temperature extremes of 5°C and 25°C. Frog tadpoles may therefore be better able to withstand lower temperatures because they spawn earlier in the year and thus their tadpoles normally experience lower temperatures than those of toads and have therefore evolved to tolerate such conditions.

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Appendix A. The mean development stage reached by upland and lowland frogs and toads after at different temperatures after a given number of days.

Time (days)	Lowland toads					Upland toads					Upland frogs					Lowland frogs				
	5°C	8°C	10°C	15°C	25°C	5°C	8°C	10°C	15°C	25°C	5°C	8°C	10°C	15°C	25°C	5°C	8°C	10°C	15°C	25°C
1	24.0	24.0	24.0	24.0	24.0	14.0	14.0	14.0	14.0	14.0	18.0	18.0	18.0	18.0	18.0					
2		24.0	24.0	24.0	24.0			14.0	14.0		18.0		18.0	18.0	18.0					
3						14.0				15.0	19.0		19.0		19.0					
4		25.0	25.0		25.0						20.0	18.0	20.0		20.0					
5				25.0			15.0	15.0	15.0	16.0	21.0		21.0		21.0					
6		26.0							16.0			19.0								
7			26.0		26.0	15.0		16.0	17.0	17.0				20.0	22.0					
8									18.0	18.0	22.0	20.0	22.0		22.0					
9		27.0			27.0	16.0			19.0	19.0				24.0	25.0					
10	25.0		27.0	27.0	28.0			17.0	20.0	20.0	23.0	21.0	23.0	26.0	27.0					
11					29.0	16.0			21.0			22.0	24.0							
12							17.0		22.0	23.0	24.0	23.0	25.0		29.0					
13													26.0			31.0	31.0	31.0	31.0	31.0
14						17.0			25.0	26.0	25.0									
15									27.0	27.0										
16										28.0		24.0								
17	26.0									29.0		25.0						31.0	31.0	
18								18.0				26.0					31.0		33.0	
19										31.0								32.0	34.0	
20					30.0					32.0							32.0	33.0	35.0	
21										32.0			26.0						36.0	
22	27.0	28.0	28.0	28.0	30.6	18.0	20.0	20.4	30.0	32.9	25.0	26.0	26.7	28.0	29.9	32.5	32.0	33.6	37.0	
23																				
24																				
25	27.2	28.0	28.5	28.5	31.6	18.0	20.2	21.0	30.0	37	26.0	26.2	27.7	28.1	31	32.7	32.6	33.9	37.4	
26	27.0	28.1	28.5	29.6	32	18.0	21.8	23.0	30.0	38.4	26.0	26.8	27.7	28.0	31.1	32.0	33.8	34.3	38.0	34.0
27	28.0	28.1	28.7	29.0	32.3	18.0	22.7	23.1	30.9	38.1	27.0	27.0	28.0	29.6	32.4	32.0	34.8	34.5	39.8	34.8
28	28.0	28.0	28.7	29.6	32.6	18.0	22.8	24.2	31.0	38.4	26.0	27.0	28.0	29.1	33.2	32.0	34.4	34.3	39.9	35.8
29	28.0	28.3	28.5	29.6	32.1	18.0	23.0	23.8	31.0	38.6	26.7	27.2	28.0	30.0	33.6	33.0	34.2	35.0	40.0	35.9
30																				
31																				
32	28.0	28.7	28.5	30.0	33.0	18.0	23.3	25.8	31.0	41.4	27.0	27.3	28.0	30.2	33.2	33.0	34.6	35.8	40.0	36.4
33	28.0	28.6	28.7	30.6	33.0	18.0	24.0	25.0	31.0	40.0	27.0	27.6	28.0	30.0	33.0	33.0	34.4	35.9	40.0	34.0
34																				
35	28.0	29.0	28.7	30.8	33.9	18.0	24.8	25.5	31.0	40.8	27.0	28.0	28.2	30.0	33.0	33.3	34.8	35.5	40.0	35.5
36	28.0	29.0	28.6	31.0	33.8	18.0	24.0	26.6	31.0	43.0	27.0	28.0	28.0	30.0	33.7	33.0	34.0	34.9	40.1	34.5
37																				
38																				
39	28.0	29.0	28.9	30.7	34.2	19.0	25.7	27.0	32.4	44	27.0	28.8	28.7	30.0	34.1	33.3	34.0	35.3	40.6	35.3
40																				
41	28.0	29.0	29.4	30.3	34.4	19.0	26.0	27.0	31.0	46.0	27.0	28.8	28.6	30.4	33.2	33.0	34.8	35.3	40.7	34.8
42	28.0	29.0	29.0	31.1	34.7	19.0	26.7	27.0	31.0		27.0	28.1	29.0	30.0	34	33.0	34.5	35.7	40.3	35.3
43	28.0	29.0	28.8	31.0	35.0	19.0	27.0	27.5	31.0		27.0	29.2	28.8	30.6	33.1	33.0	35.0	35.0	40.5	35.8
44																				
45																				
46	28.0	29.0	28.7	31.1	36	19.0	27.0	28.0	33.0		27.0	28.1	28.6	30.8	33.5	33.0	35.4	36.4	40.0	36.2
47																				
48																				
49																				
50																				
51		29.0	29.4	30.6	36	19.0	27.0	28.0	33.0		27.0	28.0	28.1	30.0	33.5	33.0	35.0	35.8	40.3	36.3
52																				
53		29.0	29.7	30.6	36	19.0	27.4	28.0	31.0		27.0	28.8	28.8	30.8	33.8	33.3	34.5	35.2	40.9	35.7
54																				
55		29.0	29.1	30.6	36	19.0	28.0	28.0	31.0		27.0	28.9	28.5	30.8	34.3	33.0	34.5	35.4	41.5	39
56																				
57			29.7	31.0	36.4	19.0	28.0	28.0	31.8		27.0	28.0	28.8	30.6	36.2	33.0	34.0	35.6	41.7	39
58																				
59																				
60			29.8	31.0	37.8	19.0	28.0	28.0	31.0		27.0	28.7	29.4	30.0	37.4	34.0	34.8	35.5	42.3	39
61																				
62			29.1	31.0	38.2	19.0	28.0	28.0	31.0		27.0	29.0	29.0	30.9	37.1	34.0	35.0	35.0	42.5	41.0
63																				
64			29.3	31.0	38.6	19.0	28.0	28.0	32.0		27.3	29.0	29.7	31.9	38	34.0	35.0	35.6	40.5	41
65																				
66																				
67			29.3	31.0	40.4	19.0	28.0	28.0	32.0		27.4	29.0	29.0	32.0	40.0	34.0	35.6	36.2	43.1	43.0
68			29.0	31.5	40.0		28.0	28.0	32.0		27.0	29.0	29.0	31.2	40.0	34.0	35.0	35.0	41.5	
69			29.1	32.8	40.2		28.0	28.0	32.0		27.0	29.0	29.0	30.3	40.8	34.0	35.0	35.0	42.2	
70																				
71			29.5	31.7	38		28.0	28.0	33.0		27.3	29.0	29.0	31.8	40.0	34.0	35.1	35.6	41.2	
72																				
73																				
74																				
75			29.2	32.5	39.3			28.0	33.0		28.0	29.0	29.0	31.2	40.8	34.0	35.0	35.0	41.4	
76			29.8	33.0	39.0			28.1	33.0		28.5	29.0	29.0	32.3	39.0	34.0	35.1	35.8	41.1	
77			29.0	33.0	39.0			28.0	33.0		28.0	29.0	29.0	32.3	39.0	34.0	35.0	35.0	41.5	
78			29.8	33.8	40.0			28.0	33.6		28.5	29.0	29.4	32.2	41.0	34.0	35.0	35.1	41.3	
79																				
80																				
81			29.5	33.0	44.0			28.0	33.0		28.6	29.0	29.5	33.0	44.0	34.0	35.0	35.3	41.0	



Appendix B. The standard error of the mean development stages reached by upland and lowland frogs and toads at different temperatures after given times.

Time (days)	Lowland toads					Upland toads					Upland frogs					Lowland frogs				
	5°C	8°C	10°C	15°C	25°C	5°C	8°C	10°C	15°C	25°C	5°C	8°C	10°C	15°C	25°C	5°C	8°C	10°C	15°C	25°C
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
2		0.00	0.00	0.00	0.00			0.00	0.00	0.00			0.00	0.00	0.00					
3			0.00			0.00				0.00				0.00	0.00					
4		0.00	0.00		0.00		0.00		0.00		0.00	0.00	0.00		0.00					
5			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.00	0.00	0.00		0.00	0.00	0.00	
6		0.00		0.00	0.00		0.00		0.00			0.00	0.00	0.00	0.00		0.00		0.00	
7			0.00		0.00	0.00		0.00		0.00			0.00	0.00	0.00		0.00		0.00	
8			0.00		0.00		0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00		0.00	
9		0.00		0.00	0.00	0.00	0.00		0.00	0.00			0.00	0.00	0.00			0.00	0.00	
10	0.00		0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
11					0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00		0.00	
12			0.00			0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.00	0.00	
13				0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14			0.00	0.00	0.00	0.00			0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00	0.00	0.00
15					0.00				0.00	0.00				0.00	0.00		0.00			
16							0.00		0.00	0.00		0.00		0.00	0.00			0.00	0.00	
17	0.00						0.00		0.00		0.00	0.00		0.00		0.00		0.00	0.00	0.00
18							0.00	0.00	0.00	0.00		0.00	0.00	0.00			0.00	0.00	0.00	0.00
19						0.00	0.00		0.00	0.00		0.00		0.00				0.00	0.00	
20					0.00			0.00		0.00		0.00	0.00				0.00	0.00	0.00	
21										0.00	0.00		0.00			0.00		0.00	0.00	
22	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.15	0.00	0.61	0.04	0.00	0.10	0.00	0.06	0.11	0.00	0.17	0.00	
23										0.00						0.00		0.00	0.00	
24					0.00					0.00						0.00	0.00	0.00	0.00	
25	0.08	0.00	0.04	0.10	0.26	0.00	0.26	0.00	0.00	0.00	0.10	0.08	0.08	0.00	0.15	0.15	0.15	0.15	0.33	
26	0.00	0.04	0.04	0.79	0.00	0.00	0.89	0.11	0.00	0.12	0.00	0.10	0.16	0.00	0.07	0.00	0.12	0.17	0.00	0.00
27	0.00	0.04	0.04	0.00	0.25	0.00	0.10	0.14	0.05	0.23	0.10	0.00	0.00	0.16	0.20	0.15	0.08	0.14	0.06	0.06
28	0.00	0.00	0.07	0.10	0.13	0.00	0.09	0.16	0.00	0.25	0.00	0.00	0.00	0.27	0.34	0.00	0.12	0.17	0.08	0.06
29	0.00	0.06	0.04	0.09	0.28	0.00	0.00	0.18	0.00	0.12	0.10	0.10	0.09	0.00	0.31	0.13	0.12	0.15	0.00	0.05
30				0.79	0.00				0.00	0.12			0.79	0.00	0.00	0.00	0.12	0.07	0.00	
31				0.00	0.25				0.05	0.23			0.00	0.05	0.16	0.06	0.25	0.23	0.20	0.06
32	0.00	0.07	0.04	0.00	0.00	0.00	0.12	0.14	0.00	8.41	0.00	0.13	0.00	0.18	0.17	0.14	0.10	0.10	0.00	2.13
33	0.00	0.09	0.04	0.08	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.12	0.15	0.00	0.00
34																				
35	0.00	0.00	0.04	0.11	0.24	0.00	0.19	0.19	0.00	0.79	0.00	0.14	0.06	0.00	0.00	0.25	0.05	0.12	0.00	0.07
36	0.00	0.00	0.04	0.00	0.24	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.13	0.05	0.14
37				0.08	0.00				0.00	0.00			0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
38																				
39	0.13	0.00	0.06	0.00	0.19	0.00	0.10	0.00	0.11	1.06	0.00	0.16	0.08	0.00	0.49	0.20	0.00	0.10	0.24	0.44
40				0.00	0.24				0.00	0.00			0.00	0.00	0.00	0.05	0.24	0.00	0.12	0.14
41	0.00	0.00	0.04	0.20	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.90	0.16	0.37	0.00	0.05	0.11	0.25	0.07
42	0.00	0.00	0.06	0.18	0.18	0.00	0.10	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00	0.25	0.10	0.11	0.13	0.19
43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.13	0.00	0.11	0.08	0.16	0.29	0.00	0.00	0.00	0.16	0.07
44																				
45				0.20	0.19				0.00	0.00			0.20	0.00	0.16	0.25	0.19	0.00	0.37	0.07
46	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.21	0.13	0.16	1.69	0.00	0.07	0.11	0.00	1.21
47				0.00	0.00				0.00				0.00	0.00	0.16	0.16	0.00		0.29	0.07
48																				
49																				
50				0.00	0.00				0.09				0.00	0.09	0.16	0.00	0.00		1.69	1.21
51	0.00	0.00	0.06	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.38	0.00	0.00	0.43	0.46	0.26
52																				
53	0.00	0.13	0.04	0.09	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.10	0.07	0.16	1.44	0.29	0.07	0.29	0.30	0.22
54																				
55	0.00	0.18	0.09	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.19	0.17	0.43	0.00	0.10	0.20	0.43	0.00
56																				
57	0.00	0.00	0.04	0.00	0.27	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.07	0.20	0.16	0.00	0.00	0.19	0.44	0.00
58																				
59				0.09	0.00				0.00				0.09	0.00	0.17	0.43	0.00		0.43	0.00
60	0.00	0.00	0.04	0.00	1.69	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.10	0.00	3.61	0.00	0.06	0.20	0.45	0.00
61				0.00	0.27				0.13				0.00	0.13	0.20	0.44	0.27		0.16	0.00
62	0.00	0.00	0.10	0.00	0.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.84	0.00	0.00	0.00	0.55	0.00
63																				
64	0.00	0.00	0.09	0.00	1.10	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.09	0.15	0.81	0.00	0.09	0.19	0.13	0.00
65																				
66				0.00	0.82				0.00				0.00	0.00	0.15	0.55	0.82		0.84	0.00
67	0.00	0.00	0.12	0.00	10.8	0.00	0.00	0.00	0.00	0.00	0.90	0.00	0.00	0.00	9.61	0.00	0.07	0.15	0.56	0.00
68	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.49	0.00
69	0.00	0.00	0.15	0.20	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	2.28	0.00	0.00	0.00	0.47	
70																				
71	0.00	0.00	0.15	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.18	0.00	0.00	0.05	0.12	0.47	0.00
72				0.18	0.00				0.16				0.18	0.16	0.59	0.49	0.00			
73				0.20	0.46				0.00				0.20	0.00	0.37	0.47	0.46		2.28	
74																				
75	0.00	0.00	0.22	0.15	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.87	0.00	0.00	0.00	0.47	
76	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.09	0.00	0.09	0.10	0.00	0.00	0.21	0.00	0.00	0.04	0.15	0.40	
77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.79	
78	0.00	0.00	0.17	0.17	0.00	0.00	0.00	0.00	0.08	0.00	0.10	0.00	0.12	0.52	0.00	0.00	0.00			

Appendix C. The population size of upland and lowland frogs and toads at different temperatures after given time intervals.

Time (days)	Lowland toads					Upland toads					Upland frogs					Lowland frogs				
	5 <sup>o</sup> c	8 <sup>o</sup> c	10 <sup>o</sup> c	15 <sup>o</sup> c	25 <sup>o</sup> c	5 <sup>o</sup> c	8 <sup>o</sup> c	10 <sup>o</sup> c	15 <sup>o</sup> c	25 <sup>o</sup> c	5 <sup>o</sup> c	8 <sup>o</sup> c	10 <sup>o</sup> c	15 <sup>o</sup> c	25 <sup>o</sup> c	5 <sup>o</sup> c	8 <sup>o</sup> c	10 <sup>o</sup> c	15 <sup>o</sup> c	25 <sup>o</sup> c
1	51	53	50	50	50	50	50	50	50	50	50	50	50	50	50					
2																				
3																				
4																				
5																				
6																				
7																				
8																				
9																				
10																				
11	40	53	50	50	48	45	50	35	49	21	50	45	50	45	44					
12	39	53	50	50	47	42	50	30	48	21	50	36	50	43	44					
13	39	53	50	50	44	42	49	28	47	21	50	34	50	41	43	50	51	50	50	50
14	37	53	50	50	41	42	48	27	47	21	50	31	50	40	41	48	51	46	50	50
15	36	53	50	49	40	42	48	26	47	21	49	29	49	40	40	46	51	44	50	50
16	36	53	50	48	38	39	47	26	47	21	48	25	48	39	39	46	51	41	50	50
17	36	53	50	47	35	36	45	26	46	21	48	22	46	37	37	46	51	39	50	50
18	35	53	49	47	33	35	45	25	45	20	48	20	46	36	36	46	51	37	50	50
19	33	53	48	45	29	35	45	23	45	20	48	19	46	36	32	45	51	34	50	50
20	31	53	48	45	27	31	43	22	45	20	48	19	44	32	31	44	51	32	50	50
21	30	53	48	45	26	27	41	22	45	20	48	19	43	28	31	43	51	28	50	50
22	30	53	48	45	24	23	37	21	44	20	47	19	40	25	30	42	51	27	50	50
23																				
24																				
25	27	53	46	43	17	16	25	20	44	19	43	19	38	14	27	42	51	25	50	49
26	25	53	46	41	16	15	25	20	43	18	40	19	36	13	23	41	51	25	50	49
27	24	53	46	40	16	11	25	20	43	18	37	19	36	12	19	41	51	24	50	49
28	23	53	46	40	16	9	25	20	43	18	37	19	36	12	19	41	51	24	50	49
29	22	53	46	37	14	6	24	20	43	18	37	19	36	12	16	40	51	22	50	49
30																				
31																				
32	20	46	46	36	14	5	22	16	43	18	36	19	33	12	15	36	51	20	50	49
33	20	44	46	35	14	5	22	16	43	16	36	18	33	12	15	34	51	20	49	49
34																				
35	20	39	45	33	14	5	22	16	43	16	36	18	33	12	15	30	51	20	48	48
36	18	38	45	33	14	5	22	16	43	16	36	18	33	12	15	29	51	20	48	47
37																				
38																				
39	15	28	44	32	9	5	22	16	43	16	36	17	33	12	15	25	51	20	48	42
40																				
41	14	25	43	30	9	5	22	16	42	15	36	16	33	12	15	25	51	20	37	33
42	13	20	42	29	9	5	22	16	42	15	36	15	33	12	15	25	51	20	34	32
43	9	20	42	28	5	5	22	16	42	15	36	15	32	12	15	25	51	20	29	27
44																				
45																				
46	6	19	42	28	5	5	21	16	40	12	36	15	32	8	15	25	51	20	29	27
47																				
48																				
49																				
50																				
51	3	16	42	28	5	5	21	16	40	8	36	15	32	8	13	21	51	20	26	23
52																				
53	0	14	38	28	5	5	21	16	40	2	35	15	30	8	10	17	51	20	26	17
54																				
55	0	9	38	28	5	5	21	16	40	2	35	15	30	8	9	17	51	20	26	11
56	0	7	37	28	5	5	21	16	40	2	35	15	30	8	9	17	51	20	26	10
57	0	5	36	28	5	5	21	16	40	2	35	15	29	8	9	17	51	20	26	9
58																				
59																				
60	0	0	33	28	5	5	21	16	40	2	35	15	28	7	8	17	51	20	26	8
61																				
62	0	0	29	24	5	5	21	16	40	2	34	15	27	7	8	16	51	20	24	8
63	0	0	27	24	5	5	21	16	40	2	34	15	27	7	8	16	51	20	22	8
64	0	0	25	24	5	5	21	16	40	2	34	15	26	7	8	16	51	20	16	8
65																				
66																				
67	0	0	18	24	5	5	21	15	39	2	32	15	26	6	8	15	51	20	14	4
68	0	0	16	24	5	5	21	15	39	2	32	15	26	6	4	14	51	20	13	3
69	0	0	14	20	5	5	21	14	38	2	32	15	26	6	4	13	51	20	13	0
70	0	0	13	19	0	5	21	14	36	2	31	15	25	6	4	12	51	20	13	0
71	0	0	12	18	5	5	21	14	36	2	31	15	24	6	4	11	51	19	13	0
72																				
73																				
74																				
75	0	0	11	13	4	5	11	13	36	2	30	15	24	6	4	8	51	19	11	0
76	0	0	8	8	3	5	0	11	36	2	29	15	24	6	3	6	51	19	8	0
77	0	0	8	8	3	5	0	11	36	2	28	15	21	6	2	6	51	19	6	0
78	0	0	8	8	3	5	0	10	36	2	27	15	18	6	2	5	51	19	6	0
79																				
80																				
81	0	0	8	8	3	5	0	10	36	2	27	15	12	6	1	4	51	19	4	0

